



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 38/18, 31/185</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/17797</b> <b>(43) International Publication Date:</b> 15 April 1999 (15.04.99)
<b>(21) International Application Number:</b> PCT/US98/21199 <b>(22) International Filing Date:</b> 6 October 1998 (06.10.98) <b>(30) Priority Data:</b> 08/946,092 6 October 1997 (06.10.97) US <b>(71) Applicant:</b> THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE [US/US]; 720 Rutland Avenue, Baltimore, MD 21205 (US). <b>(72) Inventor:</b> LEE, Se-Jin; 6711 Chokeberry Road, Baltimore, MD 21209 (US). <b>(74) Agent:</b> HAILE, Lisa, A.; Fish & Richardson P.C., Suite 1400, 4225 Executive Square, La Jolla, CA 92037 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> USE OF GROWTH DIFFERENCIATION FACTOR-9 (GDF-9) AS A CONTRACEPTIVE  <b>(57) Abstract</b>  Growth differentiation factor-9 (GDF-9) is disclosed along with its polynucleotide sequence and amino acid sequence. Also disclosed is a method for inhibiting oocyte maturation using GDF-9 inhibitors or by inducing an immune response to GDF-9 polypeptide or fragments thereof.		

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**USE OF GROWTH DIFFERENTIATION FACTOR-9 (GDF-9)**  
**AS A CONTRACEPTIVE**

**BACKGROUND OF THE INVENTION**

5   1.       *Field of the Invention*

The invention relates generally to growth factors and specifically to a new member of the transforming growth factor beta (TGF- $\beta$ ) superfamily, which is denoted, growth differentiation factor-9 (GDF-9) and methods of use as a contraceptive.

2.       *Description of Related Art*

10   The transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily encompasses a group of structurally-related proteins which affect a wide range of differentiation processes during embryonic development. The family includes, Mullerian inhibiting substance (MIS), which is required for normal male sex development (Behringer, *et al.*, *Nature*, 345:167, 1990), *Drosophila* decapentaplegic (DPP) gene product, which is required for dorsal-  
15   ventral axis formation and morphogenesis of the imaginal disks (Padgett, *et al.*, *Nature*, 325:81-84, 1987), the *Xenopus* Vg-1 gene product, which localizes to the vegetal pole of eggs ((Weeks, *et al.*, *Cell*, 51:861-867, 1987), the activins (Mason, *et al.*, *Biochem. Biophys. Res. Commun.*, 135:957-964, 1986), which can induce the formation of mesoderm and anterior structures in *Xenopus* embryos (Thomsen, *et al.*, *Cell*, 63:485,  
20   1990), and the bone morphogenetic proteins (BMPs, osteogenin, OP-1) which can induce *de novo* cartilage and bone formation (Sampath, *et al.*, *J. Biol. Chem.*, 265:13198, 1990). The TGF- $\beta$ s can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, hematopoiesis, and epithelial cell differentiation (for review, see Massague, *Cell* 49:437, 1987).

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The proteins of the TGF- $\beta$  family are initially synthesized as a large precursor protein which subsequently undergoes proteolytic cleavage at a cluster of basic residues approximately 110-140 amino acids from the C-terminus. The C-terminal regions of the proteins are all structurally related and the different family members can be classified  
5 into distinct subgroups based on the extent of their homology. Although the homologies within particular subgroups range from 70% to 90% amino acid sequence identity, the homologies between subgroups are significantly lower, generally ranging from only 20% to 50%. In each case, the active species appears to be a disulfide-linked dimer of C-terminal fragments. For most of the family members that have been studied, the  
10 homodimeric species has been found to be biologically active, but for other family members, like the inhibins (Ling, *et al.*, *Nature*, 321:779, 1986) and the TGF- $\beta$ s (Cheifetz, *et al.*, *Cell*, 48:409, 1987), heterodimers have also been detected, and these appear to have different biological properties than the respective homodimers.

The inhibins and activins were originally purified from follicular fluid and shown to have  
15 counteracting effects on the release of follicle-stimulating hormone by the pituitary gland. Although the mRNAs for all three inhibin/activin subunits (  $\alpha$ ,  $\beta$ A and  $\beta$ B) have been detected in the ovary, none of these appear to be ovary-specific (Meunier, *et al.*, *Proc.Natl.Acad.Sci. USA*, 85:247, 1988). MIS has also been shown to be expressed by granulosa cells and the effects of MIS on ovarian development have been documented  
20 both *in vivo* in transgenic mice expressing MIS ectopically (Behringer, *supra*) and *in vitro* in organ culture (Vigier, *et al.*, *Development*, 100:43, 1987).

Identification of new factors that are tissue-specific in their expression pattern will provide a greater understanding of that tissue's development and function.

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**SUMMARY OF THE INVENTION**

The present invention provides a cell growth and differentiation factor, GDF-9, a polynucleotide sequence which encodes the factor and antibodies which are immunoreactive with the factor. This factor appears to relate to various cell proliferative disorders, especially those involving ovarian tumors, such as granulosa cell tumors. GDF-9 is exclusively expressed in oocytes and plays a role in maturation of oocytes.

Thus, in one embodiment, the invention provides a method for detecting a cell proliferative disorder of ovarian origin and which is associated with GDF-9. In another embodiment, the invention provides a method of treating a cell proliferative disorder associated with abnormal levels of expression of GDF-9, by suppressing or enhancing GDF-9 activity.

In another embodiment, the invention provides a method for inhibiting oocyte maturation. The method includes contacting oocytes with an inhibiting effective amount of an inhibitor of GDF-9, thereby inhibiting oocyte maturation as compared to untreated oocytes. Inhibition of oocyte maturation provides contraception for fertile female subjects.

The invention also provides a method for inducing an immune response in a subject to GDF-9 whereby oocyte maturation is inhibited, comprising administering to a subject GDF-9 polypeptide or immunogenic fragment thereof and inducing an anti-GDF-9 immune response in the subject.

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**BRIEF DESCRIPTION OF THE DRAWINGS**

FIGURE 1 shows expression of GDF-9 mRNA in adult tissues.

FIGURES 2a-2c shows nucleotide and predicted amino acid sequence of GDF-9. Consensus N-glycosylation signals are denoted by plain boxes. The putative tetrabasic processing sites are denoted by stippled boxes. The in-frame termination codons upstream of the putative initiating ATG and the consensus polyadenylation signals are underlined. The poly A tails are not shown. Numbers indicate nucleotide position relative to the 5' end.

FIGURES 3a-3b show the alignment of the C-terminal sequences of GDF-9 with other members of the TGF- $\beta$  family. The conserved cysteine residues are shaded. Dashes denote gaps introduced in order to maximize alignment.

FIGURES 4a-4b show amino acid homologies among the different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

FIGURES 5a-5c show the immunohistochemical localization of GDF-9 protein. Adjacent sections of an adult ovary were either stained with hematoxylin and eosin (FIGURE 5a) or incubated with immune (FIGURE 5b) or pre-immune (FIGURE 5c) serum at a dilution of 1:500. Anti-GDF-9 antiserum was prepared by expressing the C-terminal portion of murine GDF-9 (residues 308-441) in bacteria, excising GDF-9 protein from preparative SDS gels, and immunizing rabbits. Sites of antibody binding were visualized using the Vectastain ABC kit (Vector Labs).

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FIGURE 6 shows a comparison of the predicted amino acid sequences of murine (top lines) and human (bottom lines) GDF-9. Numbers represent amino acid positions relative to the N-termini. Vertical lines represent sequence identities. Dots represent gaps introduced in order to maximize the alignment. The clear box shows the predicted proteolytic processing sites. The shaded boxes show the cysteine residues in the mature region of the proteins. The bars at the bottom show a schematic of the pre-(clear) and mature (shaded) regions of GDF-9 with the percent sequence identities between the murine and human sequences shown below.

FIGURES 7a-7d show *in situ* hybridization to adult ovary sections using a GDF-9 RNA probe. [<sup>35</sup>S]-labeled anti-sense (FIGURE 7a and 7c) or sense (FIGURE 7b and 7d) GDF-9 RNA probes were hybridized to adjacent paraffin-embedded sections of ovaries fixed in 4% paraformaldehyde. Sections were dipped in photographic emulsion, exposed, developed, and then stained with hematoxylin and eosin. Two representative fields are shown.

FIGURES 8a-8b show *in situ* hybridization to a postnatal day 4 ovary section using an antisense GDF-9 RNA probe. Sections were prepared as described for FIGURE 7. Following autoradiography and staining, the section was photographed under bright-field (FIGURE 8a) or dark-field (FIGURE 8b) illumination.

FIGURES 9a-9b show *in situ* hybridization to postnatal day 8 ovary sections using an antisense (FIGURE 9a) or sense (FIGURE 9b) GDF-9 RNA probe. Sections were prepared as described for FIGURE 7.

FIGURES 10a-10b show *in situ* hybridization to adult oviduct sections using an antisense (FIGURE 10a) or sense (FIGURE 10b) GDF-9 RNA probe. Sections were prepared as described for FIGURE 7.

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FIGURES 11a-11b show *in situ* hybridization to an adult oviduct (0.5 days following fertilization) section using an antisense GDF-9 RNA probe. Sections were prepared as described for FIGURE 7. Following autoradiography and staining, the section was photographed under bright-field (FIGURE 11a) or dark-field (FIGURE 11b) illumination.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a growth and differentiation factor, GDF-9 and a polynucleotide sequence encoding GDF-9. Unlike other members of the TGF- $\beta$  superfamily, GDF-9 expression is highly tissue specific, being expressed in cells primarily in ovarian tissue. In one embodiment, the invention provides a method for detection of a cell proliferative disorder of the ovary, which is associated with GDF-9 expression. In another embodiment, the invention provides a method for treating a cell proliferative disorder associated with abnormal expression of GDF-9 by using an agent which suppresses or enhances GDF-9 activity. In another embodiment, the invention provides a method for inhibiting oocyte maturation.

Due to the expression of GDF-9 in the reproductive tract, there are a variety of applications using the polypeptide, polynucleotide and antibodies of the invention, related to contraception, fertility and pregnancy. GDF-9 could play a role in regulation of the menstrual cycle and, therefore, could be useful in various contraceptive regimens.

The TGF- $\beta$  superfamily consists of multifunctional polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have regulatory, both positive and negative, effects on other peptide growth factors. The structural homology between the GDF-9 protein of this invention and the members of the TGF- $\beta$  family, indicates that GDF-9 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other



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members, it can be expected that GDF-9 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

For example, another regulatory protein that has been found to have structural homology with TGF- $\beta$  is inhibin, a specific and potent polypeptide inhibitor of the pituitary secretion of FSH. Inhibin has been isolated from ovarian follicular fluid. Because of its suppression of FSH, inhibin has potential to be used as a contraceptive in both males and females. GDF-9 may possess similar biological activity since it is also an ovarian specific peptide. Inhibin has also been shown to be useful as a marker for certain ovarian tumors (Lappohn, *et al.*, *N. Engl. J. Med.*, 321:790, 1989). GDF-9 may also be useful as a marker for identifying primary and metastatic neoplasms of ovarian origin. Similarly, GDF-9 may be useful as an indicator of developmental anomalies in prenatal screening procedures.

Another peptide of the TGF- $\beta$  family is MIS, produced by the testis and responsible for the regression of the Mullerian ducts in the male embryo. MIS has been shown to inhibit the growth of human ovarian cancer in nude mice (Donahoe, *et al.*, *Ann. Surg.*, 194:472, 1981). GDF-9 may function similarly and may, therefore, be useful as an anti-cancer agent, such as for the treatment of ovarian cancer.

GDF-9 may also function as a growth stimulatory factor and, therefore, be useful for the survival of various cell populations *in vitro*. In particular, if GDF-9 plays a role in oocyte maturation, it may be useful in *in vitro* fertilization procedures, e.g., in enhancing the success rate. Many of the members of the TGF- $\beta$  family are also important mediators of tissue repair. TGF- $\beta$  has been shown to have marked effects on the formation of collagen and causes a striking angiogenic response in the newborn mouse (Roberts, *et al.*, *Proc. Natl. Acad. Sci. USA*, 83:4167, 1986). GDF-9 may also have similar activities and may be useful in repair of tissue injury caused by trauma or burns for example.

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The term "substantially pure" as used herein refers to GDF-9 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify GDF-9 using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the GDF-9 polypeptide can also be determined by amino-terminal amino acid sequence analysis. GDF-9 polypeptide includes functional fragments of the polypeptide, as long as the activity of GDF-9 remains. Smaller peptides containing the biological activity of GDF-9 are included in the invention.

- 10 The invention provides polynucleotides encoding the GDF-9 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode GDF-9. It is understood that all polynucleotides encoding all or a portion of GDF-9 are also included herein, as long as they encode a polypeptide with GDF-9 activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides.
- 15 For example, GDF-9 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for GDF-9 also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the
- 20 invention as long as the amino acid sequence of GDF-9 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a cDNA sequence for GDF-9 which is 1712 base pairs in length and contains an open reading frame beginning with a methionine codon at nucleotide 29. The encoded polypeptide is 441 amino acids in length with a molecular weight of about 49.6 kD, as determined by nucleotide sequence analysis. The GDF-9 sequence contains a core of hydrophobic amino acids near the N-terminus, suggestive of a signal sequence for secretion. GDF-9 contains four potential N-glycosylation sites at asparagine residues 163, 229, 258, and 325 and a putative tetrabasic proteolytic

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processing site (RRRR) at amino acids 303-306. The mature C-terminal fragment of GDF-9 is predicted to be 135 amino acids in length and have an unglycosylated molecular weight of about 15.6 kD, as determined by nucleotide sequence analysis. One skilled in the art can modify, or partially or completely remove the glycosyl groups from  
5 the GDF-9 protein using standard techniques. Therefore, the functional protein or fragments thereof of the invention includes glycosylated, partially glycosylated and unglycosylated species of GDF-9.

The degree of sequence identity of GDF-9 with known TGF- $\beta$  family members ranges from a minimum of 21% with Mullerian inhibiting substance (MIS) to a maximum of  
10 34% with bone morphogenetic protein-4 (BMP-4). GDF-9 specifically disclosed herein differs from the known family members in its pattern of cysteine residues in the C-terminal region. GDF-9 lacks the fourth cysteine of the seven cysteines present in other family members; in place of cysteine at this position, the GDF-9 sequence contains a serine residue. This GDF-9 does not contain a seventh cysteine residue elsewhere in the  
15 C-terminal region.

Minor modifications of the recombinant GDF-9 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the GDF-9 polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these  
20 modifications are included herein as long as the biological activity of GDF-9 still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which are  
25 not required for GDF-9 biological activity.

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The nucleotide sequence encoding the GDF-9 polypeptide of the invention includes the disclosed sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution  
5 of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted  
10 polypeptide also immunoreact with the unsubstituted polypeptide.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences and 2) antibody screening of  
15 expression libraries to detect cloned DNA fragments with shared structural features.

Preferably the GDF-9 polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which  
20 correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a  
25 heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences

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relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete  
5 complement (Wallace, *et al.*, *Nucl. Acid Res.*, 9:879, 1981).

The development of specific DNA sequences encoding GDF-9 can also be obtained by:  
1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse  
10 transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common.  
15 This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct  
20 synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction  
25 technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences

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duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al.*, *Nucl. Acid Res.*, 11:2325, 1983).

- 5 A cDNA expression library, such as lambda gt11, can be screened indirectly for GDF-9 peptides having at least one epitope, using antibodies specific for GDF-9. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of GDF-9 cDNA.

DNA sequences encoding GDF-9 can be expressed *in vitro* by DNA transfer into a  
10 suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously  
15 maintained in the host, are known in the art.

In the present invention, the GDF-9 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the GDF-9 genetic sequences. Such expression vectors contain a  
20 promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*,  
25 *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably



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linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

Polynucleotide sequences encoding GDF-9 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms.

5 Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

Transformation of a host cell with recombinant DNA may be carried out by conventional  
10 techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the  $\text{CaCl}_2$  method using procedures well known in the art. Alternatively,  $\text{MgCl}_2$  or  $\text{RbCl}$  can be used. Transformation can also be performed after forming a protoplast of the host cell  
15 if desired.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the  
20 GDF-9 of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

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Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

- 5 The invention includes antibodies immunoreactive with GDF-9 polypeptide or functional fragments thereof. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, *et al.*, *Nature*,  
10 256:495, 1975). The term antibody as used in this invention is meant to include intact molecules as well as fragments thereof, such as Fab and F(ab')<sub>2</sub>, which are capable of binding an epitopic determinant on GDF-9.

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, for example, Green *et al.*, *Production of Polyclonal Antisera*, in IMMUNOCHEMICAL  
15 PROTOCOLS (Manson, ed.), pages 1-5 (Humana Press 1992); Coligan *et al.*, *Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters*, in CURRENT PROTOCOLS IN IMMUNOLOGY, section 2.4.1 (1992), which are hereby incorporated by reference.

The preparation of monoclonal antibodies likewise is conventional. See, for example,  
20 Kohler & Milstein, *Nature* 256:495 (1975); Coligan *et al.*, sections 2.5.1-2.6.7; and Harlow *et al.*, ANTIBODIES: A LABORATORY MANUAL, page 726 (Cold Spring Harbor Pub. 1988), which are hereby incorporated by reference. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing  
25 the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.



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Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, e.g., Coligan *et al.*, sections 2.7.1-2.7.12 and sections  
5 2.9.1-2.9.3; Barnes *et al.*, *Purification of Immunoglobulin G (IgG)*, in METHODS IN MOLECULAR BIOLOGY, VOL. 10, pages 79-104 (Humana Press 1992).

Methods of *in vitro* and *in vivo* multiplication of monoclonal antibodies is well-known to those skilled in the art. Multiplication *in vitro* may be carried out in suitable culture media such as Dulbecco's Modified Eagle Medium or RPMI 1640 medium, optionally  
10 replenished by a mammalian serum such as fetal calf serum or trace elements and growth-sustaining supplements such as normal mouse peritoneal exudate cells, spleen cells, bone marrow macrophages. Production *in vitro* provides relatively pure antibody preparations and allows scale-up to yield large amounts of the desired antibodies. Large scale hybridoma cultivation can be carried out by homogenous suspension culture in an  
15 airlift reactor, in a continuous stirrer reactor, or in immobilized or entrapped cell culture. Multiplication *in vivo* may be carried out by injecting cell clones into mammals histocompatible with the parent cells, e.g., osyngeneic mice, to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. After one to three  
20 weeks, the desired monoclonal antibody is recovered from the body fluid of the animal.

Therapeutic applications for antibodies disclosed herein are also part of the present invention. For example, antibodies of the present invention may also be derived from subhuman primate antibody. General techniques for raising therapeutically useful antibodies in baboons can be found, for example, in Goldenberg *et al.*, International  
25 Patent Publication WO 91/11465 (1991) and Losman *et al.*, *Int. J. Cancer* 46:310 (1990), which are hereby incorporated by reference.

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- A therapeutically useful anti-GDF-9 antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:3833 (1989), which is hereby incorporated in its entirety by reference. Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* 321: 522 (1986); Riechmann *et al.*, *Nature* 332: 323 (1988); Verhoeyen *et al.*, *Science* 239: 1534 (1988); Carter *et al.*, *Proc. Nat'l Acad. Sci. USA* 89: 4285 (1992); Sandhu, *Crit. Rev. Biotech.* 12: 437 (1992); and Singer *et al.*, *J. Immunol.* 150: 2844 (1993), which are hereby incorporated by reference.
- Antibodies of the invention also may be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas *et al.*, *METHODS: A COMPANION TO METHODS IN ENZYMOLOGY*, VOL. 2, page 119 (1991); Winter *et al.*, *Ann. Rev. Immunol.* 12: 433 (1994), which are hereby incorporated by reference. Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

- In addition, antibodies of the present invention may be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge.
- In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human

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antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green *et al.*, *Nature Genet.* 7:13 (1994); Lonberg *et al.*, *Nature* 368:856 (1994); and Taylor *et al.*, *Int. Immunol.* 6:579 (1994), which are hereby incorporated by reference.

- 5 Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')<sub>2</sub>. This  
10 fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. patents No. 4,036,945 and No.  
15 4,331,647, and references contained therein. These patents are hereby incorporated in their entireties by reference. *See also* Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230 (1960); Porter, *Biochem. J.* 73:119 (1959); Edelman *et al.*, *METHODS IN ENZYMOLOGY*, VOL. 1, page 422 (Academic Press 1967); and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4.
- 20 Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V<sub>H</sub> and V<sub>L</sub> chains. This  
25 association may be noncovalent, as described in Inbar *et al.*, *Proc. Nat'l Acad. Sci. USA* 69:2659 (1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. *See, e.g.*, Sandhu,

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*supra*. Preferably, the Fv fragments comprise V<sub>H</sub> and V<sub>L</sub> chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V<sub>H</sub> and V<sub>L</sub> domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which  
5 is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 97 (1991); Bird *et al.*, *Science* 242:423-426 (1988); Ladner *et al.*, U.S. patent No. 4,946,778; Pack *et al.*,  
10 *Bio/Technology* 11: 1271-77 (1993); and Sandhu, *supra*.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable  
15 region from RNA of antibody-producing cells. See, for example, Larrick *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 106 (1991).

The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both  
20 morphologically and genotypically. The GDF-9 polynucleotide that is an antisense molecule is useful in treating malignancies of the various organ systems, particularly, for example, the ovaries. Essentially, any disorder which is etiologically linked to altered expression of GDF-9 could be considered susceptible to treatment with a GDF-9 suppressing reagent.

25 The invention provides a method for detecting a cell proliferative disorder of the ovary which comprises contacting an anti-GDF-9 antibody with a cell suspected of having a GDF-9 associated disorder and detecting binding to the antibody. The antibody reactive

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with GDF-9 is labeled with a compound which allows detection of binding to GDF-9. For purposes of the invention, an antibody specific for GDF-9 polypeptide may be used to detect the level of GDF-9 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample of this invention is tissue  
5 of ovarian origin, specifically tissue containing granulosa cells or ovarian follicular fluid. The level of GDF-9 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a GDF-9-associated cell proliferative disorder. Preferably the subject is human.

In another embodiment, the invention provides a contraceptive method which includes  
10 inhibiting oocyte maturation. The method comprises contacting oocytes with an inhibiting effective amount of an inhibitor of GDF-9, thereby inhibiting oocyte maturation as compared to untreated oocytes. The term "inhibiting effective amount" as used herein means that amount of an inhibitor of GDF-9 that reduces maturation of an oocyte by about 50%, more preferably by about 75%, and most preferably by about 95%.  
15 One of skill in the art could use any of several methods for determining whether the amount of inhibitor of GDF-9 is effective. For example, maturation of an oocyte results in ovulation, therefore, prevention of ovulation is indicative of an effective amount. For a period just prior to ovulation, a female's body temperature increases, indicating a fertile period. An absence of a change in body temperature is indicative of prevention of oocyte  
20 maturation. Alternatively, one could measure the level of serum progesterone (see Example 6) in the subject prior to and following treatment with the inhibitor of GDF-9, where a low level of progesterone maintained throughout the period is indicative of inhibition of maturation. Levels of luteinizing hormone (LH) generally increase at the time of ovulation as well, and continuous low levels of LH can serve as an indicator of  
25 inhibition of maturation of oocytes as well. Other events associated with ovulation can be monitored in a similar manner and will be known to those of skill in the art.

An "inhibitor of GDF-9" as used herein, refers to GDF-antibodies or fragments thereof as described above, antisense molecules, or peptide or peptidomimetics of GDF-9

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polypeptide, for example. Other small molecules or chemical compounds may also be GDF-9 inhibitors. Also included are compounds identified using combinatorial chemistry methods for identifying chemical compounds that bind to GDF-9 or GDF-9 receptor. Preferably, when the inhibitor is a GDF-9 antibody or fragment thereof, the  
5 antibody is administered in a dose range of 0.1 ug to 100 mg. Preferably, the inhibitor is administered *in vivo* and preferably, the subject is a mammal, such as a human. The term "contacting" refers to administering the GDF-9 inhibitor in such a way that the inhibitor inhibits GDF-9. Methods of administration are described in detail below.

The method of the invention is also useful for inducing an immune response in a subject  
10 to GDF-9 whereby oocyte maturation is inhibited, comprising administering to a subject GDF-9 polypeptide or immunogenic fragment thereof and inducing an anti-GDF-9 immune response in the subject. Methods of producing immunogenic fragments of a polypeptide are well known in the art.

Polypeptides or peptides, including GDF-9 variants useful for the present invention  
15 comprise analogs, homologs, muteins and mimetics of GDF-9 that retain the ability to induce a specific immune response to GDF-9 or to inhibit oocyte maturation. Peptides of GDF-9 refer to portions of the amino acid sequence of GDF-9 that also retain this ability. The variants can be generated directly from GDF-9 itself by chemical modification, by proteolytic enzyme digestion, or by combinations thereof. Additionally,  
20 genetic engineering techniques, as well as methods of synthesizing polypeptides directly from amino acid residues, can be employed.

Peptides of the invention can be synthesized by such commonly used methods as t-BOC or FMOC protection of alpha-amino groups. Both methods involve stepwise syntheses whereby a single amino acid is added at each step starting from the C terminus of the  
25 peptide (See, Coligan, *et al.*, *Current Protocols in Immunology*, Wiley Interscience, 1991, Unit 9). Peptides of the invention can also be synthesized by the well known solid phase peptide synthesis methods described Merrifield, *J. Am. Chem. Soc.*, 85:2149,



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1962), and Stewart and Young, *Solid Phase Peptides Synthesis*, (Freeman, San Francisco, 1969, pp.27-62), using a copoly(styrene-divinylbenzene) containing 0.1-1.0 mMol amines/g polymer. On completion of chemical synthesis, the peptides can be deprotected and cleaved from the polymer by treatment with liquid HF-10% anisole for about 1/4-1  
5 hours at 0°C. After evaporation of the reagents, the peptides are extracted from the polymer with 1% acetic acid solution which is then lyophilized to yield the crude material. This can normally be purified by such techniques as gel filtration on Sephadex G-15 using 5% acetic acid as a solvent. Lyophilization of appropriate fractions of the column will yield the homogeneous peptide or peptide derivatives, which can then be  
10 characterized by such standard techniques as amino acid analysis, thin layer chromatography, high performance liquid chromatography, ultraviolet absorption spectroscopy, molar rotation, solubility, and quantitated by the solid phase Edman degradation. Alternatively, peptides can be produced by recombinant methods.

The term "substantially purified" as used herein refers to a molecule, such as a peptide  
15 that is substantially free of other proteins, lipids, carbohydrates, nucleic acids, and other biological materials with which it is naturally associated. For example, a substantially pure molecule, such as a polypeptide, can be at least 60%, by dry weight, the molecule of interest. One skilled in the art can purify GDF-9 peptides using standard protein purification methods and the purity of the polypeptides can be determined using standard  
20 methods including, *e.g.*, polyacrylamide gel electrophoresis (*e.g.*, SDS-PAGE), column chromatography (*e.g.*, high performance liquid chromatography (HPLC)), and amino-terminal amino acid sequence analysis.

Non-peptide compounds that mimic the binding and function of GDF-9 ("mimetics") can be produced by the approach outlined in Saragovi *et al.*, *Science* 253: 792-95 (1991).  
25 Mimetics are molecules which mimic elements of protein secondary structure. See, for example, Johnson *et al.*, "Peptide Turn Mimetics," in BIOTECHNOLOGY AND PHARMACY, Pezzuto *et al.*, Eds., (Chapman and Hall, New York 1993). The underlying rationale behind the use of peptide mimetics is that the peptide backbone of

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proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions. For the purposes of the present invention, appropriate mimetics can be considered to be the equivalent of GDF-9 itself.

Longer peptides can be produced by the "native chemical" ligation technique which links  
5 together peptides (Dawson, *et al.*, *Science*, 266:776, 1994). Variants can be created by recombinant techniques employing genomic or cDNA cloning methods. Site-specific and region-directed mutagenesis techniques can be employed. See CURRENT PROTOCOLS IN MOLECULAR BIOLOGY vol. 1, ch. 8 (Ausubel *et al.* eds., J. Wiley & Sons 1989 & Supp. 1990-93); PROTEIN ENGINEERING (Oxender & Fox eds., A.  
10 Liss, Inc. 1987). In addition, linker-scanning and PCR-mediated techniques can be employed for mutagenesis. See PCR TECHNOLOGY (Erich ed., Stockton Press 1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vols. 1 & 2, *supra*. Protein sequencing, structure and modeling approaches for use with any of the above techniques are disclosed in PROTEIN ENGINEERING, *loc. cit.*, and CURRENT PROTOCOLS IN  
15 MOLECULAR BIOLOGY, vols. 1 & 2, *supra*.

The invention also includes various pharmaceutical compositions that prevent maturation of an oocyte. The pharmaceutical compositions according to the invention are prepared by bringing an antibody against GDF-9, a peptide or peptide derivative of GDF-9, a GDF-9 mimetic, or a GDF-9-binding agent according to the present invention into a form  
20 suitable for administration to a subject using carriers, excipients and additives or auxiliaries. Frequently used carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols.  
25 Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in *Remington's*



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*Pharmaceutical Sciences*, 15th ed. Easton: Mack Publishing Co., 1405-1412, 1461-1487 (1975) and *The National Formulary XIV.*, 14th ed. Washington: American Pharmaceutical Association (1975), the contents of which are hereby incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See  
5 *Goodman and Gilman's The Pharmacological Basis for Therapeutics* (7th ed.).

The methods involves administering to a subject a therapeutically or inhibiting effective dose of a pharmaceutical composition containing the compounds of the present invention and a pharmaceutically acceptable carrier. "Administering" the pharmaceutical  
10 composition of the present invention may be accomplished by any means known to the skilled artisan. By "subject" is meant any mammal, preferably a human.

The pharmaceutical compositions are preferably prepared and administered in dose units. Solid dose units are tablets, capsules and suppositories. For treatment of a patient, depending on activity of the compound, manner of administration, nature and severity  
15 of the disorder, age and body weight of the patient, different daily doses are necessary. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals.

20 The pharmaceutical compositions according to the invention are in general administered topically, intravenously, orally or parenterally or as implants, but even rectal use is possible in principle. Suitable solid or liquid pharmaceutical preparation forms are, for example, granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, aerosols, drops or injectable solution in ampule  
25 form and also preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are

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customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of present methods for drug delivery, see Langer, *Science*, 249: 1527-1533 (1990), which is incorporated herein by reference.

- 5 The pharmaceutical compositions according to the invention may be administered locally or systemically. By "therapeutically effective dose" is meant the quantity of a compound according to the invention necessary to prevent, to cure or at least partially arrest the symptoms of the disease and its complications. An "inhibiting effective amount" of GDF-9 inhibitor has been defined above for use as in contraception regimes. Amounts  
10 effective for this use will, of course, depend on the severity of the disease and the weight and general state of the patient. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of particular disorders. Various considerations are described, *e.g.*, in Gilman  
15 *et al.* (eds.) (1990) GOODMAN AND GILMAN'S: THE PHARMACOLOGICAL BASES OF THERAPEUTICS, 8th ed., Pergamon Press; and REMINGTON'S PHARMACEUTICAL SCIENCES, 17th ed. (1990), Mack Publishing Co., Easton, Pa., each of which is herein incorporated by reference.

The pharmaceutical compositions of the invention may also be delivered by a slow  
20 release vehicle. Compounds of the invention can be further diluted by addition of suspending medium or other biologically acceptable carrier to obtain injectable or implantable slow release formulations of any therapeutically effective total dosage. One approach which has been used to provide controlled release compositions for drug delivery is liposome encapsulation. Among the main types of liposomes, multivesicular  
25 liposomes (Kim, *et al.*, *Biochim. Biophys. Acta*; 728:339-348, 1983), are uniquely different from unilamellar liposomes (Huang, *Biochemistry*; 8:334-352, 1969; Kim, *et al.*, *Biochim. Biophys. Acta*; 646:1-10, 1981), multilamellar liposomes (Bangham, *et al.*, *J. Mol. Bio.*, 13:238-252, 1965), and stable plurilamellar liposomes (U.S. Patent No.

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4,522,803). U.S. Patent No. 5,077,056 discloses studies that show the rate of release of the encapsulated biological agent from liposomes into an aqueous environment can be modulated by introducing protonophores or ionophores into liposomes to create a membrane potential. In addition, a method is known (U.S. Patent No. 5,186,941) for  
5 controlling the release rate of drugs from vesicle compositions wherein the liposomes containing a therapeutic agent encapsulated are suspended in a solution containing sufficient solute to provide an osmolarity substantially isotonic with respect to that of the solution within the vesicles, and hypertonic with respect to physiological saline. In multivesicular liposomes, it is also known (WO 96/08253) to control the rate of release  
10 of active agents by introducing an osmotic spacer into the aqueous solution in which the active agent is dissolved prior to formation of the multivesicular liposomes.

The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized  
15 in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay.  
20 Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

25 The antibodies of the invention can be bound to many different carriers and used to detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and

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magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill  
5 in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

10 Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific antihapten antibodies.

15 In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having the antigen comprising a polypeptide of the invention for which the monoclonal antibodies  
20 are specific.

The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to  
25 give the best target-to-background signal ratio.

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As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

- 5 For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a  
10 particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to  
15 immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentaacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are  $^{111}\text{In}$ ,  $^{97}\text{Ru}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{72}\text{As}$ ,  $^{89}\text{Zr}$ , and  $^{201}\text{Tl}$ .

The monoclonal antibodies of the invention can also be labeled with a paramagnetic  
20 isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include  $^{157}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ ,  $^{52}\text{Cr}$ , and  $^{56}\text{Fe}$ .

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- The monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a GDF-9-associated disease in a subject. Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes in the concentration of such antigen present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the GDF-9-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the GDF-9-associated disease in the subject receiving therapy. Similarly, such techniques can be used to follow a contraceptive regime in a subject.
- 10 The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal cell, therefore, it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Thus, where a cell-proliferative disorder is associated with the expression of GDF-9, nucleic acid sequences that interfere with GDF-9 expression at the translational level can be used.
- 15 This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific GDF-9 mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme. In addition, antisense molecules or ribozymes are useful in the contraceptive methods of the invention where it is desirable to inhibit GDF-9 expression. As a further mechanism of action, in "transcription arrest"
- 20 it appears that some polynucleotides can form "triplex," or triple-helical structures with double stranded genomic DNA containing the gene of interest, thus interfering with transcription by RNA polymerase. Giovannangeli *et al.*, *Proc. Natl. Acad. Sci.* 90:10013 (1993); Ebbinghaus *et al. J. Clin. Invest.* 92:2433 (1993).

- Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded.



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Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target GDF-9-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal. Biochem.*, 172:289,  
5 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and  
10 cleave it (Cech, *J. Amer. Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize  
15 sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences  
20 are preferable to shorter recognition sequences.

The present invention also provides gene therapy for the treatment of cell proliferative disorders which are mediated by GDF-9 protein or where it is desirable to inhibit GDF-9 expression for purposes of contraception. Such therapy would achieve its therapeutic effect by introduction of the GDF-9 antisense polynucleotide into cells having the  
25 proliferative disorder or introduction of antisense into oocytes. Delivery of antisense or sense GDF-9 polynucleotide, GDF-9 polypeptide or peptides can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system.

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Especially preferred for therapeutic delivery of antisense sequences is the use of targeted liposomes.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus.

- 5 Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these
- 10 vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF-9 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a sugar, a glycolipid,
- 15 or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing the GDF-9 antisense polynucleotide.

- 20 Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA
- 25 transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include, but are not limited to  $\Psi$ 2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are



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replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag*, *pol* and *env*, by conventional  
5 calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for GDF-9 polynucleotides or polypeptides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule  
10 complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0  $\mu\text{m}$  can encapsulate a  
15 substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al.*, *Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an  
20 efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic  
25 information (Mannino, *et al.*, *Biotechniques*, 6:682, 1988).

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The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of  
5 divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms,  
10 particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished  
15 based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a  
20 monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the  
25 lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

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The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

### EXAMPLE 1

#### 5                    IDENTIFICATION AND ISOLATION OF A NOVEL                          TGF- $\beta$ FAMILY MEMBER

To identify a new member of the TGF- $\beta$  superfamily, degenerate oligonucleotides were designed which corresponded to two conserved regions among the known family members: one region spanning the two tryptophan residues conserved in all family  
10 members except MIS and the other region spanning the invariant cysteine residues near the C-terminus. These primers were used for polymerase chain reactions on mouse genomic DNA followed by subcloning the PCR products using restriction sites placed at the 5' ends of the primers, picking individual *E. coli* colonies carrying these subcloned inserts, and using a combination of random sequencing and hybridization analysis to  
15 eliminate known members of the superfamily.

GDF-9 was identified from a mixture of PCR products obtained with the primers SJL160 (5'-CCGGAATTCGGITGG(G/C/A)A(G/A/T/C)(G/C/A)A(G/A/T/C) TGG(A/G)TI(A/-G)TI(T/G)CICC-3') and SJL153 (5'-CCGGAATTC(A/G)CAI(G/C)C-(A/G)CAIC(T/C)(G/A/T/C)(C/G/T)TIG(T/C)I(G/A)(T/C)CAT-3'). PCR using these  
20 primers was carried out with 2  $\mu$ g mouse genomic DNA at 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min for 40 cycles.

PCR products of approximately 280 bp were gel-purified, digested with Eco RI, gel-purified again, and subcloned in the Bluescript vector (Stratagene, San Diego, CA). Bacterial colonies carrying individual subclones were picked into 96 well microtiter  
25 plates, and multiple replicas were prepared by plating the cells onto nitrocellulose. The

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replicate filters were hybridized to probes representing known members of the family, and DNA was prepared from non-hybridizing colonies for sequence analysis.

The primer combination of SJL160 and SJL153, yielded three known sequences (inhibin  $\beta$ B, BMP-2, and BMP-4) and one novel sequence (designated GDF-9) among 145  
5 subclones analyzed.

RNA isolation and Northern analysis were carried out as described previously (Lee, S.J., *Mol. Endocrinol.* 4:1034, 1990). An oligo dT-primed cDNA library was prepared from 2.5-3  $\mu$ g of ovary poly A-selected RNA in the lambda ZAP II vector according to the instructions provided by Stratagene. The ovary library was not amplified prior to  
10 screening. Filters were hybridized as described previously (Lee, S.-J., *Proc. Natl. Acad. Sci. USA.*, 88:4250-4254, 1991). DNA sequencing of both strands was carried out using the dideoxy chain termination method (Sanger, *et al.*, *Proc. Natl. Acad. Sci., USA*, 74:5463-5467, 1977) and a combination of the S1 nuclease/exonuclease III strategy (Henikoff, S., *Gene*, 28:351-359, 1984) and synthetic oligonucleotide primers.

15

## EXAMPLE 2

### EXPRESSION PATTERN AND SEQUENCE OF GDF-9

To determine the expression pattern of GDF-9, RNA samples prepared from a variety of adult tissues were screened by Northern analysis. Five micrograms of twice polyA-selected RNA prepared from each tissue were electrophoresed on formaldehyde gels,  
20 blotted and probed with GDF-9. As shown in Figure 1, the GDF-9 probe detected a 1.7 kb mRNA expressed exclusively in the ovary.

A mouse ovary cDNA library of  $1.5 \times 10^6$  recombinant phage was constructed in lambda ZAP II and screened with a probe derived from the GDF-9 PCR product. The nucleotide sequence of the longest of nineteen hybridizing clones is shown in Figure 2. Consensus  
25 N-glycosylation signals are denoted by plain boxes. The putative tetrabasic processing

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sites are denoted by stippled boxes. The in-frame termination codons upstream of the putative initiating ATG and the consensus polyadenylation signals are underlined. The poly A tails are not shown. Numbers indicate nucleotide position relative to the 5' end. The 1712 bp sequence contains a long open reading frame beginning with a methionine codon at nucleotide 29 and potentially encoding a protein 441 amino acids in length with a molecular weight of 49.6 kD. Like other TGF- $\beta$  family members, the GDF-9 sequence contains a core of hydrophobic amino acids near the N-terminus suggestive of a signal sequence for secretion. GDF-9 contains four potential N-glycosylation sites at asparagine residues 163, 229, 258, and 325 and a putative tetrabasic proteolytic processing site (RRRR) at amino acids 303-306. The mature C-terminal fragment of GDF-9 is predicted to be 135 amino acids in length and have an unglycosylated molecular weight of 15.6 kD. Although the C-terminal portion of GDF-9 clearly shows homology with the other family members, the sequence of GDF-9 is significantly diverged from those of the other family members (Figures 3 and 4). Figure 3 shows the alignment of the C-terminal sequences of GDF-9 with the corresponding regions of human GDF-1 (Lee, *Proc. Natl. Acad. Sci. USA*, 88:4250-4254, 1991), *Xenopus* Vg-1 (Weeks, *et al.*, *Cell*, 51:861-867, 1987), human Vgr-1 (Celeste, *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human OP-1 (Ozkaynak, *et al.*, *EMBO J.*, 9:2085-2093, 1990), human BMP-5 (Celeste, *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), *Drosophila* 60A (Wharton, *et al.*, *Proc. Natl. Acad. Sci. USA*, 88:9214-9218, 1991), human BMP-2 and 4 (Wozney, *et al.*, *Science*, 242:1528-1534, 1988), *Drosophila* DPP (Padgett, *et al.*, *Nature*, 325:81-84, 1987), human BMP-3 (Wozney, *et al.*, *Science*, 242:1528-1534, 1988), human MIS (Cate, *et al.*, *Cell*, 45:685-698, 1986), human inhibin ,  $\beta$ A, and  $\beta$ B (Mason, *et al.*, *Biochem. Biophys. Res. Commun.*, 135:957-964, 1986), human TGF- $\beta$ 1 (Derynck, *et al.*, *Nature*, 316:701-705, 1985), human TGF- $\beta$ 2 (deMartin, *et al.*, *EMBO J.*, 6:3673-3677, 1987), human TGF- $\beta$ 3 (ten Dijke, *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:4715-4719, 1988), chicken TGF- $\beta$ 4 (Jakowlew, *et al.*, *Mol. Endocrinol.*, 2:1186-1195, 1988), and *Xenopus* TGF- $\beta$ 5 (Kondaiah, *et al.*, *J. Biol. Chem.*, 265:1089-1093, 1990). The conserved cysteine residues are shaded. Dashes denote gaps introduced in order to maximize the alignment.

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Figure 4 shows the amino acid homologies among the different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

- 5 The degree of sequence identify with known family members ranges from a minimum of 21% with MIS to a maximum of 34% with BMP-4. Hence, GDF-9 is comparable to MIS in its degree of sequence divergence from the other members of this superfamily. Moreover, GDF-9 shows no significant sequence homology to other family members in the pro-region of the molecule. GDF-9 also differs from the known family members in  
10 its pattern of cysteine residues in the C-terminal region. GDF-9 lacks the fourth cysteine of the seven cysteines that are present in all other family members; in place of cysteine at this position, the GDF-9 sequence contains a serine residue. In addition, GDF-9 does not contain a seventh cysteine residue elsewhere in the C-terminal region.

### EXAMPLE 3

#### 15 IMMUNOCHEMICAL LOCALIZATION OF GDF-9 IN THE ZONA PELLUCIDA

To determine whether GDF-9 mRNA was translated, sections of adult ovaries were incubated with antibodies directed against recombinant GDF-9 protein. In order to raise antibodies against GDF-9, portions of GDF-9 cDNA spanning amino acids 30 to 295  
20 (pro-region) or 308 to 441 (mature region) were cloned into the T7-based pET3 expression vector (provided by F.W. Studier, Brookhaven National Laboratory), and the resulting plasmids were transformed into the BL21 (DE3) bacterial strain. Total cell extracts from isopropyl  $\beta$ -D-thiogalactoside-induced cells were electrophoresed on SDS/polyacrylamide gels, and the GDF-9 protein fragments were excised, mixed with  
25 Freund's adjuvant, and used to immunize rabbits by standard methods known to those of skill in the art. All immunizations were carried out by Spring Valley Lab (Sykesville, MD). The presence of GDF-9 reactive antibodies in the sera of these rabbits were assessed by Western analysis of bacterially-expressed protein by Western analysis.



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For immunohistochemical studies, ovaries were removed from adult mice, fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. Sites of antibody binding were detected by using the Vectastain ABC kit, according to the instructions provided by Vector Laboratories. FIGURE 5 shows the immunohistochemical localization of GDF-9 protein. Adjacent sections of an adult ovary were either stained with hematoxylin and eosin (FIGURE 5a) or incubated with immune (FIGURE 5b) or pre-immune (FIGURE 5c) serum at a dilution of 1:500. As shown in FIGURE 5b, the antiserum detected protein solely in oocytes. No staining was detected using pre-immune serum (FIGURE 5c). Hence, GDF-9 protein appears to be translated *in vivo* by oocytes.

10

**EXAMPLE 4****ISOLATION OF HUMAN GDF-9**

In order to isolate a cDNA clone encoding human GDF-9, a cDNA library was constructed in lambda ZAP II using poly A-selected RNA prepared from an adult human ovary. From this library, a cDNA clone containing the entire human GDF-9 coding sequence was identified using standard screening techniques as in Example 1 and using the murine GDF-9 clone as a probe. A comparison of the predicted amino acid sequences of murine (top lines) and human (bottom lines) GDF-9 is shown in FIGURE 6. Numbers represent amino acid positions relative to the N-termini. Vertical lines represent sequence identities. Dots represent gaps introduced in order to maximize the alignment. The clear box shows the predicted proteolytic processing sites. The shaded boxes show the cysteine residues in the mature region of the proteins. The bars at the bottom show a schematic of the pre-(clear) and mature (shaded) regions of GDF-9 with the percent sequence identities between the murine and human sequences shown below.

Like murine GDF-9, human GDF-9 contains a hydrophobic leader sequence, a putative RXXR proteolytic cleavage site, and a C-terminal region containing the hallmarks of other TGF- $\beta$  family members. Murine and human GDF-9 are 64% identical in the pro-region and 90% identical in the predicted mature region of the molecule. The high degree

25

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of homology between the two sequences suggest that human GDF-9 plays an important role during embryonic development and/or in the adult ovary.

### **EXAMPLE 5**

#### **NUCLEIC ACID DETECTION OF EXPRESSION OF GDF-9 IN OOCYTES**

5 In order to localize the expression of GDF-9 in the ovary, *in situ* hybridization to mouse ovary sections was carried out using an antisense GDF-9 RNA probe. FIGURE 7 shows *in situ* hybridization to adult ovary sections using a GDF-9 RNA probe. [<sup>35</sup>S]-labeled anti-sense (FIGURE 7a and 7c) or sense (FIGURE 7b and 7d) GDF-9 RNA probes were hybridized to adjacent paraffin-embedded sections of ovarians fixed in 4%  
10 paraformaldehyde. Sections were dipped in photographic emulsion, exposed, developed, and then stained with hematoxylin and eosin. Two representative fields are shown.

As shown in FIGURES 7a and 7c, GDF-9 mRNA was detected primarily in oocytes in adult ovaries. Every oocyte (regardless of the stage of follicular development) examined showed GDF-9 expression, and no expression was detected in any other cell types. No  
15 hybridization was seen using a control GDF-9 sense RNA probe (FIGURE 7b and 7d). Hence, GDF-9 expression appears to be oocyte-specific in adult ovaries.

To determine the pattern of expression of GDF-9 mRNA during ovarian development, sections of neonatal ovaries were probed with a GDF-9 RNA probe. FIGURE 8 shows *in situ* hybridization to a postnatal day 4 ovary section using an antisense GDF-9 RNA  
20 probe. Sections were prepared as described for FIGURE 7. Following autoradiography and staining, the section was photographed under bright-field (FIGURE 8a) or dark-field (FIGURE 8b) illumination.

FIGURE 9 shows *in situ* hybridization to postnatal day 8 ovary sections using an antisense (FIGURE 9a) or sense (FIGURE 9b) GDF-9 RNA probe. Sections were  
25 prepared as described for FIGURE 7.



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GDF-9 mRNA expression was first detected at the onset of follicular development. This was most clearly evident at postnatal day 4, where only oocytes that were present in follicles showed GDF-9 expression (FIGURE 8): no expression was seen in oocytes that were not surrounded by granulosa cells. By postnatal day 8, every oocyte appeared to  
5 have undergone follicular development, and every oocyte showed GDF-9 expression (FIGURE 9).

To determine whether GDF-9 was also expressed following ovulation, sections of mouse oviducts were examined by *in situ* hybridization. FIGURE 10 shows *in situ* hybridization to adult oviduct sections using an antisense (FIGURE 10a) or sense  
10 (FIGURE 10b) GDF-9 RNA probe. Sections were prepared as described for FIGURE 7.

FIGURE 11 shows *in situ* hybridization to adult oviduct (0.5 days following fertilization) section using an antisense GDF-9 RNA probe. Sections were prepared as described for FIGURE 7. Following autoradiography and staining, the section was photographed  
15 under bright-field (FIGURE 11a) or dark-field (FIGURE 11b) illumination.

As shown in FIGURE 10, GDF-9 was expressed by oocytes that had been released into the oviduct. However, the expression of GDF-9 mRNA turned off rapidly following fertilization of the oocytes; by day 0.5 following fertilization, only some embryos (such as the one shown in FIGURE 11) expressed GDF-9 mRNA, and by day 1.5, all embryos  
20 were negative for GDF-9 expression.

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**EXAMPLE 6**  
**CONTRACEPTIVE TARGET**

- GDF-9 is a novel protein growth factor which regulates the early development and the maturation of the egg (oocyte). Experimental results demonstrate that this factor is essential to the development of the egg. Several lines of evidence support this including:
- 5 1) GDF-9 mRNA is expressed exclusively by developing and maturing oocytes; 2) GDF-9 protein is secreted by maturing oocytes and is essential for the normal development of the follicle; and 3) GDF-9 deficient (knockout) female mice do not develop mature eggs or follicles and are completely sterile (Dong *et al.*, *Nature*, 383,531, 1996).
- 10 GDF-9 is a highly specific and novel target for the development of a female contraceptive or as a nonsurgical method of sterilization. There are several different approaches one may use to develop a contraceptive using GDF-9 as a target: 1) the use of the GDF-9 protein or a peptide fragment as a "vaccine"; 2) use of an anti-GDF-9 antibody which binds to and effectively inhibit or neutralize the biological actions of the
- 15 GDF-9 molecule; and 3) the use of a GDF-9 receptor antagonist. The term "vaccine" as used herein refers to a composition or compound that induces a protective immune response. For example, a GDF-9 "vaccine" protects against conception or pregnancy and acts as a contraceptive.

- The following examples include different ways that oocyte development and
- 20 maturation may be regulated in order to prevent ovulation or to cause permanent, nonsurgical sterilization in humans and in other species. These examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

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### 1. Active Immunization: GDF-9 "vaccines"

The intact GDF-9 protein, or a fragment thereof, is an effective source of the composition used to induce an immune response directed toward oocytes (*e.g.*, vaccine). The intact GDF-9 protein or a monomeric subunit can be economically produced in mammalian cell  
5 culture, yeast or microbial culture (such as *Bacillus* or *E. coli*). Alternatively, a peptide fragment of GDF-9 is also a suitable source for producing a vaccine. Typically, such fragments are 10 to 30 amino acids long, are exposed on the exterior portions of the GDF-9 molecule, and may be located within or near the receptor binding portion of the GDF-9 molecule.

10 Several methods can be used to enhance the antigenicity of the GDF-9, or a fragment thereof. Such methods are well known and include forming a chemical complex between a hapten (GDF-9 or a fragment thereof) and a larger carrier molecule (hapten-carrier); mixing the antigen with an adjuvant; or denaturing the protein with heat, detergent, or chaotropic agent (urea or guanidine-HCl).

15 The following experiment can be performed to demonstrate that a GDF-9 composition is an effective contraceptive. Using New Zealand white rabbits as a model, the rabbits are immunized with either the intact GDF-9, a GDF-9 monomer, or one of the GDF-9 fragments listed in the table below. The route of administration is subcutaneous when an adjuvant is used. In the case when an adjuvant is not used, the routes of  
20 administration may include subcutaneous, intramuscular, intravenous or intraperitoneal. The preferred route is subcutaneous.

Typically, single or multiple immunization may be required to produce an immune response to GDF-9. The humoral immune response may be monitored by routine serologic methods. Briefly, one to two weeks following the first immunization, a serum  
25 sample is obtained and the antibody titer to the GDF-9 protein is determined by either

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**ELISA or RIA methods. A second or third immunization may be required every 10-14 days in order to produce an effective immune response.**

Table 1

GDF-9 vaccines, proteins, concentrations and formulations

	Vaccine	Protein concentration	Formulation
		(mg)	
5	Intact GDF-9 homodimer	0.1, 1.0, 10	neutral buffered saline solution with or without adjuvant <sup>1</sup>
	Heat denatured or chemically denatured GDF-9	0.1, 1.0, 10	neutral buffered saline solution with or without adjuvant
	GDF-9 monomer	0.1, 1.0, 10	neutral buffered saline solution with or without adjuvant
10	GDF-9 monomer conjugated to carrier protein <sup>2</sup>	0.1, 1.0, 10	neutral buffered saline solution with or without adjuvant
	GDF-9 fragments 1, 2 or 3 (predicted exposed site within putative receptor binding domain)	0.1, 1.0, 10	neutral buffered saline solution with adjuvant
15	GDF-9 fragments 1, 2 or 3 conjugated to a carrier protein	0.1, 1.0, 10	neutral buffered saline solution with or without adjuvant

<sup>1</sup>Adjuvants suitable for use in animals include Freund's complete or incomplete adjuvant. Adjuvants suitable for use in humans will be known to those of skill in the art.

<sup>2</sup>Monomer is covalently coupled to a suitable carrier protein such as heat denatured immunoglobulin, denatured albumin or KLH.

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Rabbits are a good model to demonstrate the effectiveness of a GDF-9 vaccine. Rabbits are immunized with GDF-9 or a GDF-9 fragment in an adjuvant. Antibody titers to GDF-9 are determined in order to assure that a humoral response has been achieved. Gonadotrophin is administered in order to induce ovulation in the rabbit. Following  
5 gonadotrophin administration serum progesterone levels are measured. Low serum progesterone levels are an indication that ovulation is inhibited by hyperimmunizing the rabbits with GDF-9 vaccines.

Histologic evaluation of the ovaries from GDF-9 vaccinated rabbits will show that the ovaries are intact. A mild mixed focal inflammatory infiltrate within the medulla of each  
10 ovary is observed. The ovaries will be devoid of mature oocytes and follicles. The cortex will appear normal. Histologic examination of the fallopian tubes and uterus will reveal no abnormalities or inflammation.

## 2. Passive Immunization

Passive immunization is a second approach to developing a GDF-9 specific  
15 contraceptive. Using this approach, antibodies are produced which have high affinity of GDF-9. The antibodies are purified by conventional affinity chromatography methods (e.g., protein-A). In this instance, either a immunoglobulin enriched serum fraction (gamma globulin) or a monoclonal antibody generated against GDF-9 is suitable. In these instances, the anti-GDF-9 gamma globulin or the monoclonal antibody will have  
20 the property of specifically binding to GDF-9. The antibody may either block the binding of the GDF-9 to its natural receptor or function to increase the rate of clearance of the GDF-9 from the ovary. In either case, the objective is to allow the antibody to contact the GDF-9 produced by the oocytes and prevent it from contacting its natural receptor.

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For use in humans, the preferred antibody is a humanized monoclonal antibody. Production of murine monoclonal antibodies is well established and familiar to those skilled in the art. Briefly, mice are immunized with GDF-9 homodimer, monomer or a fragment (Table 1). Once a humoral response is produced, the spleen or lymph node  
5 cells are removed and fused with a mouse myeloma cell, typically SP2/0 or P-2 cells. The fused cells (hybridoma) are cultured in a selection media, cloned and subcloned to generate an immortal cell line producing an antibody to GDF-9.

Due to its immunogenicity, the murine monoclonal antibody is not most desirable to use as a therapeutic for human use. The antigenicity of a murine antibody can be reduced by  
10 replacing the murine immunoglobulin heavy and light chain constant domains with the equivalent human antibody domains (chimeric antibody). The chimeric antibody can be further "humanized" by replacing intervening structural murine sequences within the antibodies hypervariable regions. This is referred to as a "fully humanized" antibody. The fully humanized antibody is preferred since this antibody can be used repeatedly in  
15 a human subject without causing an immune response to the antibody.

### 3. GDF-9 Receptor Antagonist

A third approach to suppressing GDF-9 function is to develop a receptor antagonist. The approach is to first identify cell surface receptor(s) which are responsible for signal transduction in the ovary. These receptors are likely to be expressed on the egg or  
20 associated accessory cells (*e.g.*, granulosa or thecal). Once a target cell population is identified, a receptor binding assay is used to identify peptides or small organic compounds which will bind to the GDF-9 receptor and prevent the GDF-9 from binding to its receptor. The use of the receptor antagonist has the advantage of being a specific inhibitor of GDF-9 induction of oocyte maturation and the potential to be a long-term,  
25 reversible therapeutic.



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Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

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### SUMMARY OF SEQUENCES

Sequence ID No. 1 is the nucleotide sequence for the primer, SJL160, for GDF-9 (page 24, lines 15 and 16);

Sequence ID No. 2 is the nucleotide sequence for the primer, SJL153, for GDF-9 (page 5 24, lines 17 and 18);

Sequence ID No. 3 is the nucleotide and deduced amino acid sequence for GDF-9 (Figure 2);

Sequence ID No. 4 is the deduced amino acid sequence for GDF-9 (Figure 2);

Sequence ID No. 5 is the amino acid sequence of the C-terminus of GDF-3 (Figure 3);

10 Sequence ID No. 6 is the amino acid sequence of the C-terminus of GDF-9 (Figure 3);

Sequence ID No. 7 is the amino acid sequence of the C-terminus of GDF-1 (Figure 3);

Sequence ID No. 8 is the amino acid sequence of the C-terminus of Vg-1 (Figure 3);

Sequence ID No. 9 is the amino acid sequence of the C-terminus of Vgr-1 (Figure 3);

Sequence ID No. 10 is the amino acid sequence of the C-terminus of OP-1 (Figure 3);

15 Sequence ID No. 11 is the amino acid sequence of the C-terminus of BMP-5 (Figure 3);

Sequence ID No. 12 is the amino acid sequence of the C-terminus of 60A (Figure 3);

Sequence ID No. 13 is the amino acid sequence of the C-terminus of BMP-2 (Figure 3);

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Sequence ID No. 14 is the amino acid sequence of the C-terminus of BMP-4 (Figure 3);

Sequence ID No. 15 is the amino acid sequence of the C-terminus of DPP (Figure 3);

Sequence ID No. 16 is the amino acid sequence of the C-terminus of BMP-3 (Figure 3);

Sequence ID No. 17 is the amino acid sequence of the C-terminus of MIS (Figure 3);

5 Sequence ID No. 18 is the amino acid sequence of the C-terminus of inhibin (Figure 3);

Sequence ID No. 19 is the amino acid sequence of the C-terminus of  $\beta$ A (Figure 3);

Sequence ID No. 20 is the amino acid sequence of the C-terminus of  $\beta$ B (Figure 3);

Sequence ID No. 21 is the amino acid sequence of the C-terminus of TGF- $\beta$ 1 (Figure 3);

Sequence ID No. 22 is the amino acid sequence of the C-terminus of TGF- $\beta$ 2 (Figure 3);

10 Sequence ID No. 23 is the amino acid sequence of the C-terminus of TGF- $\beta$ 3 (Figure 3);

Sequence ID No. 24 is the amino acid sequence of the C-terminus of TGF- $\beta$ 4 (Figure 3);

Sequence ID No. 25 is the amino acid sequence of the C-terminus of TGF- $\beta$ 5 (Figure 3);

and

Sequence ID No. 26 is the amino acid sequence of the C-terminus of GDF-9 (Figure 6).

**CLAIMS**

1. A method for inhibiting oocyte maturation comprising contacting oocytes with an inhibiting effective amount of an inhibitor of GDF-9, thereby inhibiting oocyte maturation as compared to untreated oocytes.
2. The method of claim 1, wherein the inhibitor is a GDF-9 antibody or fragment thereof.
3. The method of claim 2, wherein the antibody is a monoclonal antibody.
4. The method of claim 3, wherein the antibody is a humanized antibody.
5. The method of claim 2, wherein the antibody is administered to a subject *in vivo*.
6. The method of claim 2, wherein the antibody is administered in a dose range of about 0.1 ug to 100 mg.
7. The method of claim 1, wherein the inhibitor is a GDF-9 antisense molecule.
8. A method for inducing an immune response in a subject to GDF-9 whereby oocyte maturation is inhibited, comprising administering to a subject GDF-9 polypeptide or immunogenic fragment thereof and inducing an anti-GDF-9 immune response in the subject.
9. The method of claim 8, wherein the GDF-9 polypeptide or fragment thereof is administered with an adjuvant.
10. The method of claim 8, wherein the GDF-9 polypeptide or fragment thereof is administered by injection or by implantation in a slow release delivery vehicle.

11. The method of claim 8, wherein the GDF-9 polypeptide or fragment thereof is administered in a dose range of about 0.1 ug to 100 mg.

ADRENAL  
PANCREAS  
INTESTINE  
SPLEEN  
KIDNEY  
LUNG  
HEART  
BRAIN  
OVARY  
LIVER  
THYMUS  
UTERUS  
SEMINAL VESICLE  
TESTIS  
10.5 d PLACENTA

- 1.7 kb



FIG. 1



1 ATGCGTTCCTTCTTAGTTCCTCCAGTCAATGGCACTTCCAGCAACTTCCCTGTTCGGGGT 60  
61 TTGCTGCTTGGCTGGCTGTGTTTCTTAGTAGCCCTTAGCTCTCAGGCTTCTACTGAAGA 120  
C C F A W L C F L S S L S S Q A S T E E  
121 ATCCAGAGTGGAGCCAGTGAATAATGTGAGTCTGAGGCAGACCCCTGGTCCTTGCTGCT 180  
S Q S G A S E N V E S E A D P W S L L L  
181 GCCTGTAGATGGGACTGACAGGTCTGGCCTCTTGGCCCCCTCTTTAAGGTTCTATCTGA 240  
P V D G T D R S G L L P P L F K V L S D  
241 TAGGCGAGGTGAGACCCCTAAGCTGCAGCCTGACTCCAGAGCACTCTACTACATGA AAA 300  
R R G E T P K L Q P D S R A L Y Y M K K  
301 GCTCTATAAGACGTATGCTACCAAGAGGGGGTTCCTCCAAACCCAGCAGAAAGTCACCTCTA 360  
L Y K T Y A T K E G V P K P S R S H L Y  
361 CAATACCGTCCGGCTCTTCAGTCCCCTGTGCCAGCAAGAGCAGGCCACCCAGCAACCAGGT 420  
N T V R L F S P C A Q Q E Q A P S N Q V  
421 GACAGGACCGCTGCCGATGGTGACCTGCTGTTTAACCTGGACCGGTGACTGCCATGGA 480  
T G P L P M V D L L F N L D R V T A M E  
481 AACTTGCCTCAAATCGGCTCTTGCTATACACTCTGAACAACCTTGCCTCTTCCCTCCAC 540  
H L L K S V L L Y T L N N S A S S S T  
541 TGTGACCTGTATGTGACCTTGTGGTAAAGGAGGCCCATGTCTTCTGCGAGGGCACCCCC 600  
V T C M C D L V V K E A M S S G R A P P

FIG.2a

601 AAGAGCACCGTACTCATTCACCCCTGAAGAAACACAGATGGATTGAGATTGATGTGACCTC 660  
 R A P Y S F T L K K H R W I E I D V T S  
 661 CCTCCTTCAGCCCCCTAGTGACCTCCAGCGAGAGCATTCACCTGTCTGTCAATTTTAC 720  
 L L Q P L V T S S E R S I H L S V **N F T**  
 721 ATGCACAAAGACCAGGTGCCAGAGGACGAGTGTTTAGCATGCCCTCTCTCAGTGCCTCC 780  
 C T K D Q V P E D G V F S M P L S V P P  
 781 TTCCCTCATCTTGATCTCAACGACACAAGCACCCAGCCCTACCACCTCTTGGCAGTCTCT 840  
 S L I L Y L **N D T** S T Q A Y H S W Q S L  
 841 TCAGTCCACCTGGAGGCCCTTTACAGCATCCCGCCAGCCGGTGTGGCTGCCCGTCCCCGT 900  
 Q S T W R P L Q H P G Q A G V A A R P V  
 901 GAAAGAGGAAGCTACTGAGGTGGAAAGATCTCCCCGGCCGTCGAGGGCAGAAAGCCAT 960  
 K E E A T E V E R S P **R R R R** G Q K A I  
 961 CCGCTCCGAAGCGAAGGGCCACTTCTTACAGCATCCTTCAACCTCAGCGAATACTTCAA 1020  
 R S E A K G P L L T A S F **N L S** E Y F K  
 1021 ACAGTTTCTTTTCCCCCAAAACGAGTGCTGAACCTCCATGACTTCAGACTGAGTTTGTAGTCA 1080  
 Q F L F P Q N E C E L H D F R L S F S Q  
 1081 GCTCAAAATGGACAACCTGGATCGTGGCCCCCGACAGGTACAAACCCTAGGTACTGTAAAGG 1140  
 L K W D N W I V A P H R Y N P R Y C K G

3/15

FIG.2b

1141 GGACTGTCCCTAGGGCGGTCAGGCATCGGTATGGCTCTCCTGTGCACACCATCGTCCAGAA 1200  
D C P R A V R H R Y G S P V H T M V Q N  
1201 TATAATCTATGAGAAGCTGGACCCCTTCAGTCCCAAGGCCCTTCGTGTGTCGGCGCAAGTA 1260  
I I Y E K L D P S V P R P S C V P G K Y  
1261 CAGCCCCCTGAGTGTGTGACCATTGAACCCGACGGCTCCATCGCTTACAAGAGTACGA 1320  
S P L S V L T I E P D G S I A Y K E Y E  
1321 AGACATGATAGCTACGAGGTGCACCTGTCTGTTAGCATGGGGGCCACTTCAACAAGCCCTGC 1380  
D M I A T R C T C R \*  
1381 CTGGCAGAGCAATGCTGTGGGCCCTTAGAGTGCCTCGGCAGAGAGCTTCCCTGTGACCAGTC 1440  
1441 TCTCCGTGCTGCTCAGTGCACACTGTGTGAGCGGGGAGTGTGTGTGTGATGACCA 1500  
1501 CATCGAGTGCAGTGTCCGTAGGTGTAAGGGCACACTGCTCGTTGCCATAAACCAA 1560  
1561 GTGAAATGTAACCTCATTTGGAGAGCTCTTTCTCCCCACGAGTGTACTTTTTCAGTGGACAG 1620  
1621 ATTTGTTAGCATAAGTCTCGAGTAGAATGTAGCTGTGAACATGTCACAGTGTGTGCTTTT 1680  
1681 TATGTGACGGAAGAATAAACTGTTCATGCCAT 1712

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FIG.2c

GDF-3	KRRAAISVPKGFC--RNFCRRHQLFNF-QDLGWHKWVIAPKGFMANYPCHGECPFSMTTYLNS--
GDF-9	FNLSEYFKQFLFP--QNECELHDFRLSF-SQLKWDNWIVAPHRYNPRYCKGDCPPRAVRHRYGS--
GDF-1	PRRDAEPVLGGGP--CGACRRARRLYVSF-REVGWHRWVIAPRGFLANYCQGCALPVALSGSGGP
Vg-1	RRKRSYSKLPFTA--SNICKKRHLHYVEF-KDVGWQNWNVIAPQGYMANYPYGECPYPLTEILNG--
Vgr-1	RVSSASDYNSSSEL--KTACKRKHELHYVSF-QDLGWQDWIIAPKGYAANYCDGECSFPLNAHMNA--
OP-1	RMANVAENSSSDQ--RQACKKHHELHYVSF-RDLGWQDWIIAPEGYAAYYCEGECAFPLNSYMNA--
BMP-5	RMSSVGDYNTSEQ--KQACKKHHELHYVSF-RDLGWQDWIIAPEGYAAFYCDGECSFPLNAHMNA--
60A	SPNNVPLLEPMES--TRSCQMQLTYIDF-KDLGWHDWIIAPEGYCAFYCSGEONFPLNAHMNA--
BMP-2	EKRQAKHKQRKRL--KSSCKRHPLVDF-SDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNS--
BMP-4	RSPKHHSQARKK--NKNCRRHSLYVDF-SDVGWNDWIVAPPGYQAFYCHGDCPPFPLADHLNS--
DPP	KRHARRPTRRKNH--DDTORRHSLYVDF-SDVGWDDWIVAPLGYDAYYCHGKCPFPLADHFNS--
BMP-3	QTLKKARRKQWIE--PRNCARRYLKVDF-ADIGWSEWIIISPKSFDAYYCSGACQFPMPSLKP--
MIS	PGRAQRSAGATAA--DGPCALRELSVDL---RAERSVLIPETYQANNQGVCGWPQSDRNPRY--
Inhibin α	LRLLQRPPEEPAA--HANCHRVALNISF-QELGWERWIVYPPSFIFHYCHGCGLHIPPNLSLPV
Inhibin βA	RRRRRGLECDGKV--NICKCKQFFVSF-KDIGWNDWIIAPSGYHANYCEGECPSHIAGTSGSSL
Inhibin βB	RIRKRGLECDGRT--NLCCRQQFFIDF-RLIGWNDWIIAPTGYGNYCEGSCPAYLAGVPGSAS
TGF-β1	RRALDTNYCFSST--EKNCCVRQLYIDFRKDLGWK-WIHEPKGYHANFCLGCPYIWSLD-----
TGF-β2	KRALDAAYCFRNV--QDNCCLRPLYIDFKRDLGWK-WIHEPKGYNANFCAACPYLWSSD-----
TGF-β3	KRALDTNYCFRNL--EENCCVRPLYIDFRQDLGWK-WVHEPKGYANFCSGCPYLRSD-----
TGF-β4	RRDLDTDYCFPGTDEKNCCVRPLYIDFRKDLQWK-WIHEPKGYMANFOMGFCPYIWSAD-----
TGF-β5	KRGVGGQEYCFGNN--GPNCCVKPLYINFRKDLGWK-WIHEPKGYEANYCLGNCPYIWSMD-----

FIG.3a

GDF-3	--SNYAFMQALMHM--	--ADPKVPKAVCN--	--PTKLSPISMLYQ--	DSDKNVILRHYEDMVVDE	CGG
GDF-9	--PVHTMVQNI IYE--	--KLDPSPVPRPSCV--	--PGKYSPLSVLTI--	EPDGS IAYKEYEDMIATRC	IOR
GDF-1	PALNHAVLRLMHA	--AAPGAADLECCV--	--PARLSPISVLFF--	DNSDNVVLQRQYEDMVVDE	CCOR
Vg-1	--SNHAILQTLVHS--	--IEPEDIPLPCCV--	--PTKMSPISMLFY--	DNNDNVVLRRHYENMAVDE	CCOR
Vgr-1	--TNHAI VQTLVHL--	--MNPEYVPKPCCA--	--PTKLNAISVLYF--	DDNSNVILKKYRNMMVVR	ACCH
OP-1	--TNHAI VQTLVHF--	--INPETVPKPCCA--	--PTQLNAISVLYF--	DDSSNVILKKYRNMMVVR	ACCH
BMP-5	--TNHAI VQTLVHL--	--MFPDHVPKPCCA--	--PTKLNAISVLYF--	DDSSNVILKKYRNMMVVR	ACCH
60A	--TNHAI VQTLVHL--	--LEPKKVPKPCCA--	--PTRLGALPVLHY--	LDENNVNLKKYRNMI VKS	CCCH
BMP-2	--TNHAI VQTLVNS--	--VNSKIPKACCV--	--PTELSAISMLYL--	DENEKVVLKNYQDMVVE	CCOR
BMP-4	--TNHAI VQTLVNS--	--VNSSI PKACCV--	--PTELSAISMLYL--	DEYDKVVLKNYQEMVVE	CCOR
DPP	--TNHAVVQTLVNN--	--MNPGKVPKACCV--	--PTQLDSVAMLYL--	NDQSTVVLKNYQEMTVV	CCOR
BMP-3	--SNHATI QSI VRA--	--VGVPGIPPCCV--	--PEKMSSLSILFF--	DENKNVVLKVYPNMTVES	CCOR
MIS	--GNHVLL LKMQA--	--RGAALARPCCV--	--PTAYAGKLLISLSEER--	ISAHVVPNMTVATE	CCOR
Inhibin $\alpha$	--PGAPPTPAQPYS--	--LLPGAQPCCAALPGTMRPLHVR	TTSDGGYSFKYETVPNLLTQH	ACCI	
Inhibin $\beta A$	--SFHSTVINHYRMRGHSPFANLKS	CCV--	--PTKL RPMSMLY--	DDGQNI IKKDI QNMIVEE	CCOS
Inhibin $\beta B$	--SFHTAVVNQYRMRLNPGT--	VNSCCI--	--PTKLSTMSMLYF--	DDEYNIVKRDVPNMIVEE	CCCA
TGF- $\beta 1$	--TQYSKVLALYNQ--	--HNPGASAAPCCV--	--PQALEPLPIVY--	VGRKPKV-EQLSNMIVRS	CKOS
TGF- $\beta 2$	--TQHSRVLSLYNT--	--INPEASASPCCV--	--SQDLEPLTILY--	IGKTPKI-EQLSNMIVKS	CKOS
TGF- $\beta 3$	--TTHSTVLGLYNT--	--LNPEASASPCCV--	--PQDLEPLTILY--	VGRTPKV-EQLSNMIVKS	CKOS
TGF- $\beta 4$	--TQYTKVLALYNQ--	--HNPGASAAPCCV--	--PQTLDPPLPIIY--	VGRNVRV-EQLSNMIVRA	CKOS
TGF- $\beta 5$	--TQYSKVLSLYNQ--	--NNPGASISPCCV--	--PDVLEPLPIIY--	VGR TAKV-EQLSNMIVRSC	NCS

FIG.3b





[illegible]

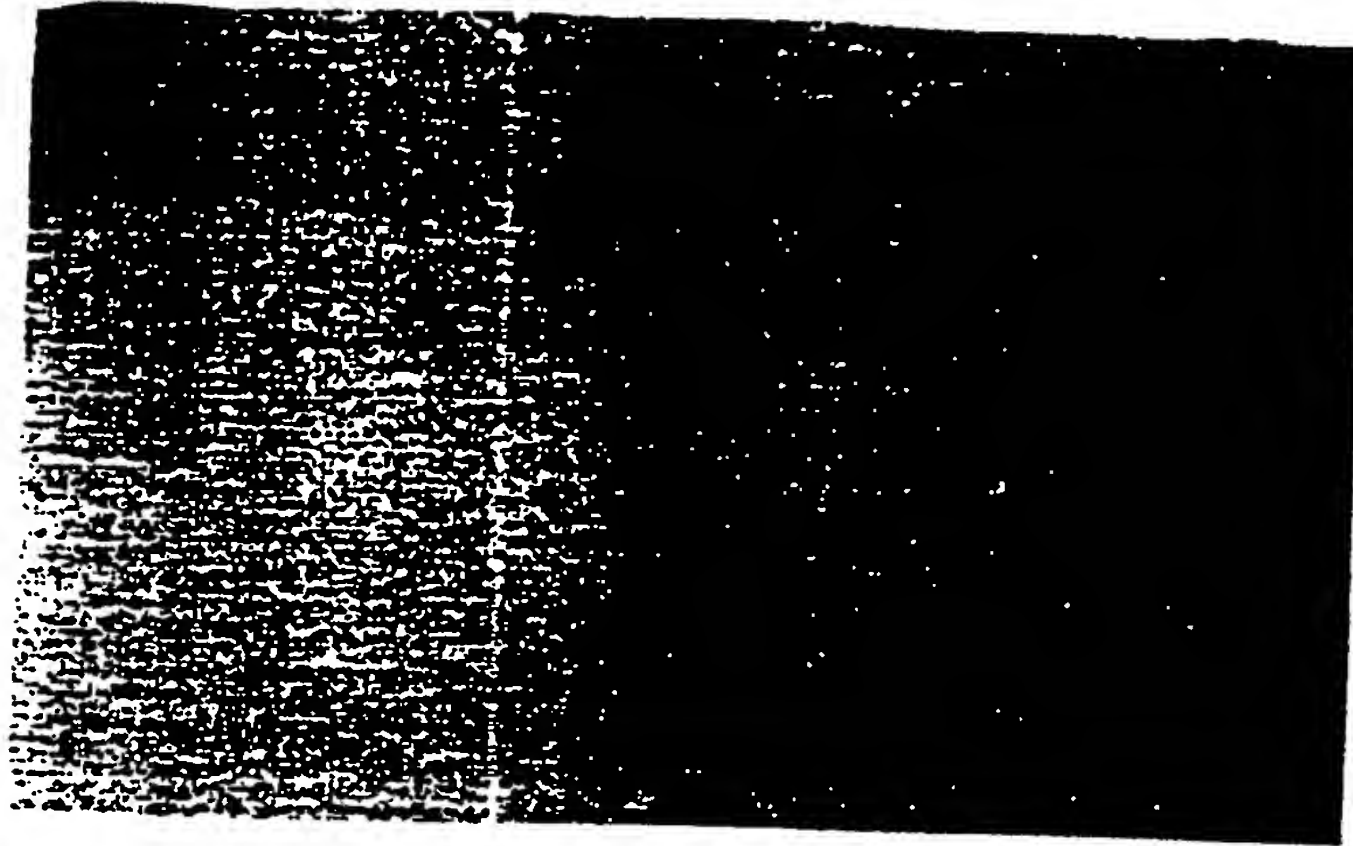


FIG. 5c

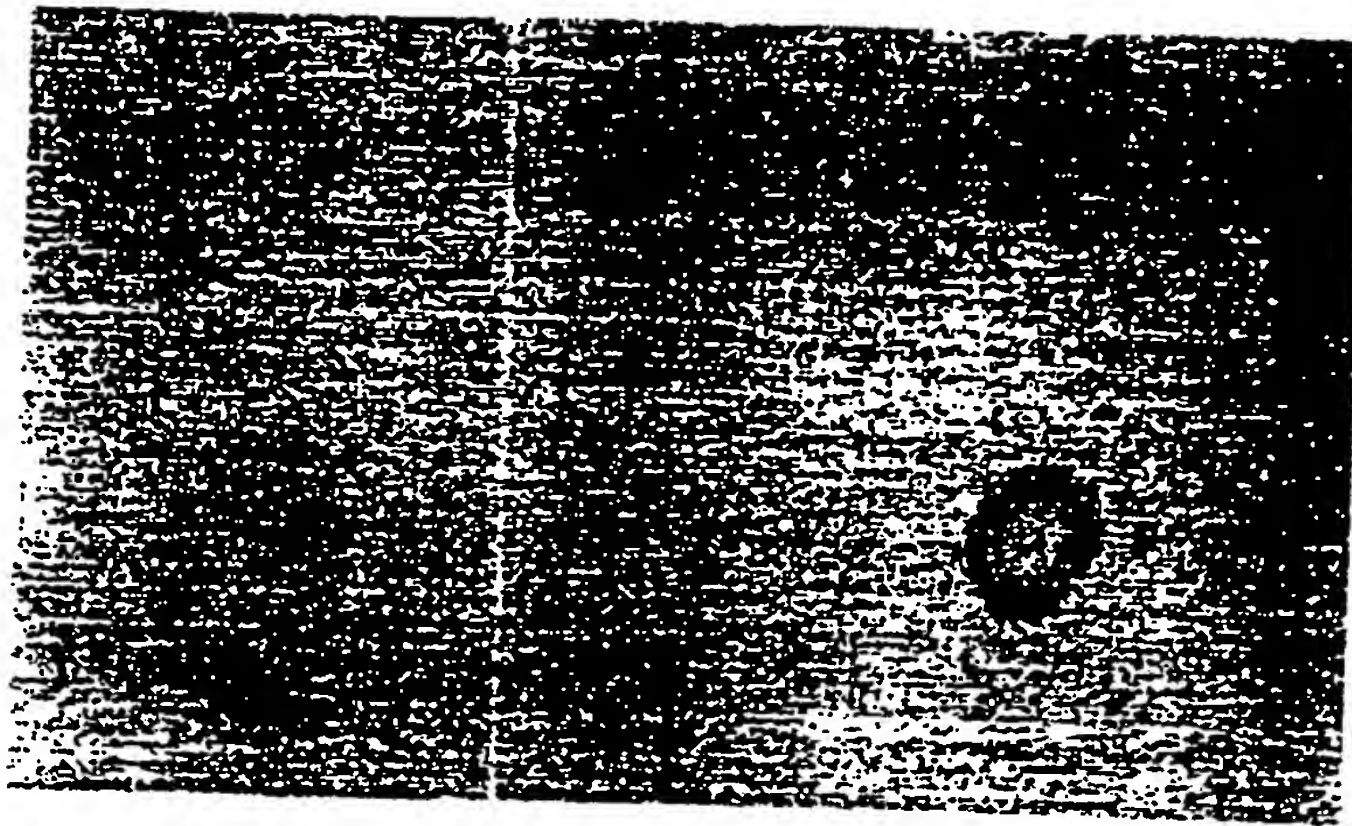


FIG. 5b

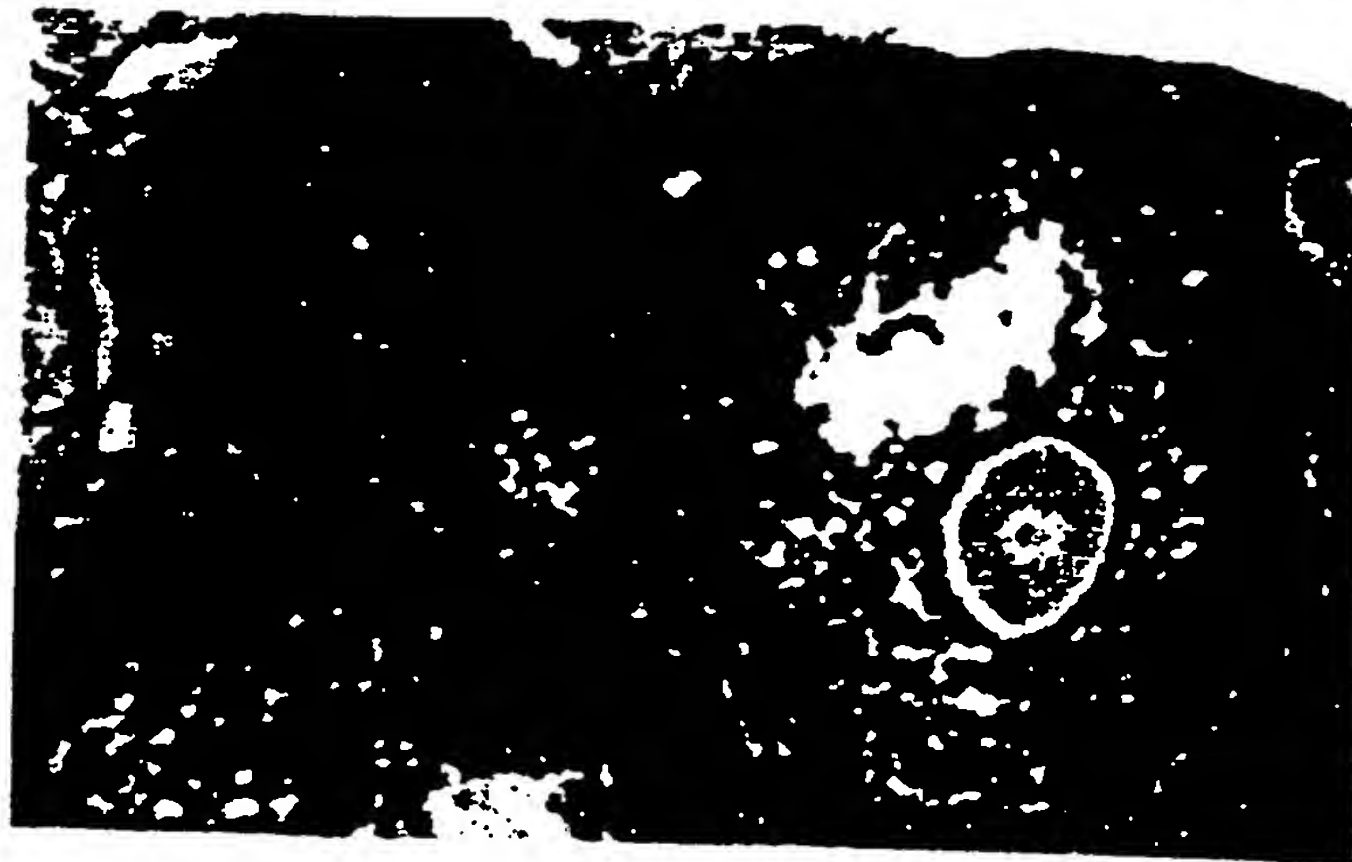


FIG. 5a

10/15

1 MALPSNFLLGVCCFAWLCFLSSLSSQASTEESQSGASENVESEADPWSLL 50  
 || | ||| ||||| || ||| | | ||| |||  
 1 MARPNKFLWFCFAWLCFPISLGSQASGGEAQIAASAELESGAMPWSLL 50  
  
 51 LPVDGTDRSGLLPPLFKVLSDRRGETPKLQPDSTRALYYMKKLYKTYATKE 100  
 | || ||| ||||| || | ||||| ||||| |||||  
 51 QHIDERDRAGLLPALFKVLSVGRGGSPLQPDSTRALHYMKKLYKTYATKE 100  
  
 101 GVPKPSRSHLYNTVRLFSPCAQQEQAPSNQVTGPLPMVDLLFNLDRTAM 150  
 | || ||||| ||| || ||| ||| ||| |||  
 101 GIPKSNRSHLYNTVRLFTPCTRHKQAPGDQVTGILPSVELLFNLDRTTV 150  
  
 151 EHLLKSVLLYTLNNSASSSSTVTCMCDLVVKEAMSSGRAPPRAPYSFTL 199  
 ||||| ||| ||| ||| ||| ||| ||| |||  
 151 EHLLKSVLLYNINNSVSFSSAVKVCNLMIKEPKSSSRTLGRAPYSFTFN 200  
  
 200 .....KKHRWIEIDVTSLLQPLVTSSERSIHLSVNFTCTKDQV....PE 239  
 ||| || ||||| ||| | ||| | ||| |||  
 201 SQFEFGKKHKWIIQIDVTSLLQPLVASNKRSIHMSINFTCMKDQLEHPSAQ 250  
  
 240 DGVFSMPLSVPPSLILYLNDTSTQAYHSWQSLQSTWRPLQHPGQA.GVAA 283  
 | | | | ||||| ||||| || | | | |  
 251 NGLFNMTL.VSPSLILYLNDTSAQAYHSWYSLHYKRRPSQGPDOERSLSA 299  
  
 289 RPYKEEATEVERSP...RRRGQKAIKSEAKGPLLTASFNLSEYFKQFLFP 336  
 || ||| | || |||| | | ||| ||||| |||  
 300 YPVGEEAAEDGRSSHRRRGQETVSSELKKPLGPASFNLSEYFRQFLLP 349  
  
 337 QNECELHDFRLSFSQLKWDNWIVAPHRYNPRYCKGDEPRAVRHRYGSPVH 386  
 ||||| ||||| ||||| ||||| ||||| ||||| |||||  
 350 QNECELHDFRLSFSQLKWDNWIVAPHRYNPRYCKGDEPRAVGHRYGSPVH 399  
  
 387 TMVQNIIEKLDPSVPRPSVPGKYSPLSVLTIEPDGSIAYKEYEDMIAT 436  
 ||||| ||||| ||||| ||||| ||||| ||||| |||||  
 400 TMVQNIIEKLDSSVPRPSVPAKYSPLSVLTIEPDGSIAYKEYEDMIAT 449  
  
 437 RQTER 441  
 |||||  
 450 KQTER 454  
 |||||



64%

FIG. 6

90%

FIG. 7a

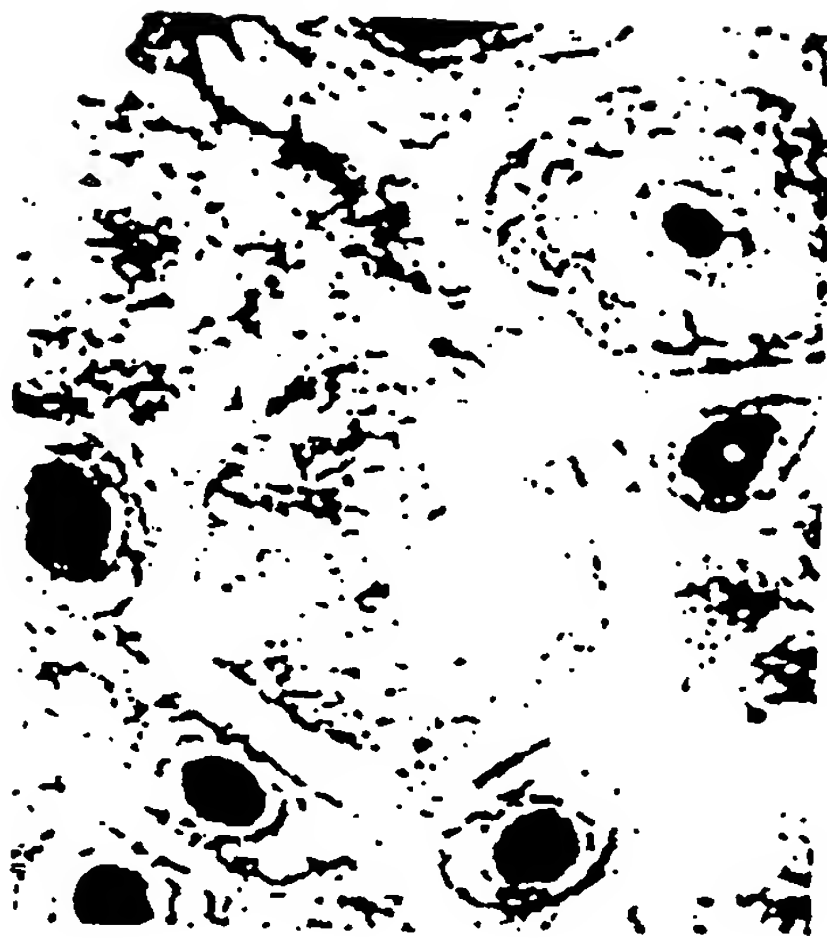


FIG. 7b



FIG. 7c



FIG. 7d



FIG. 8a



FIG. 8b



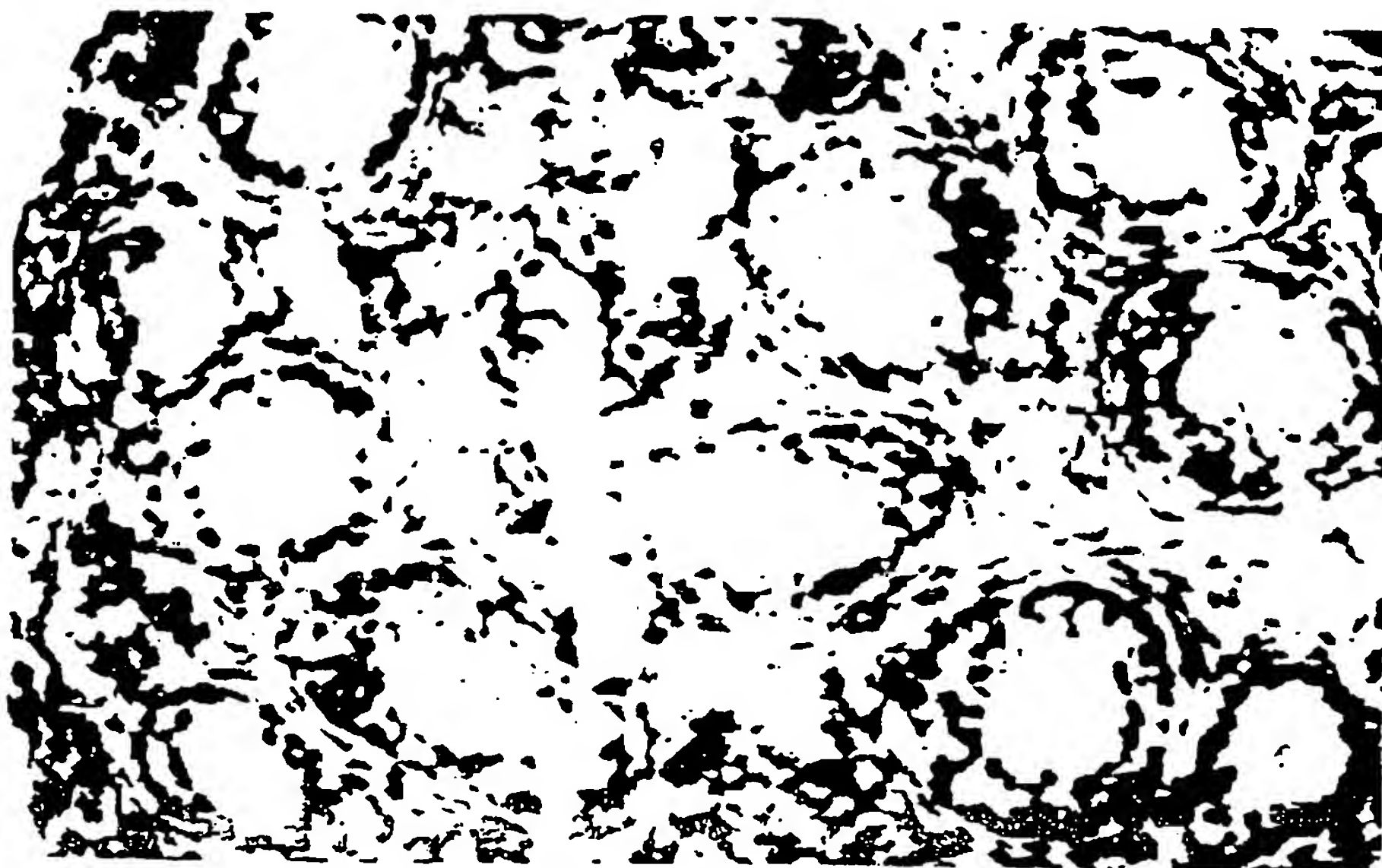


FIG. 9b

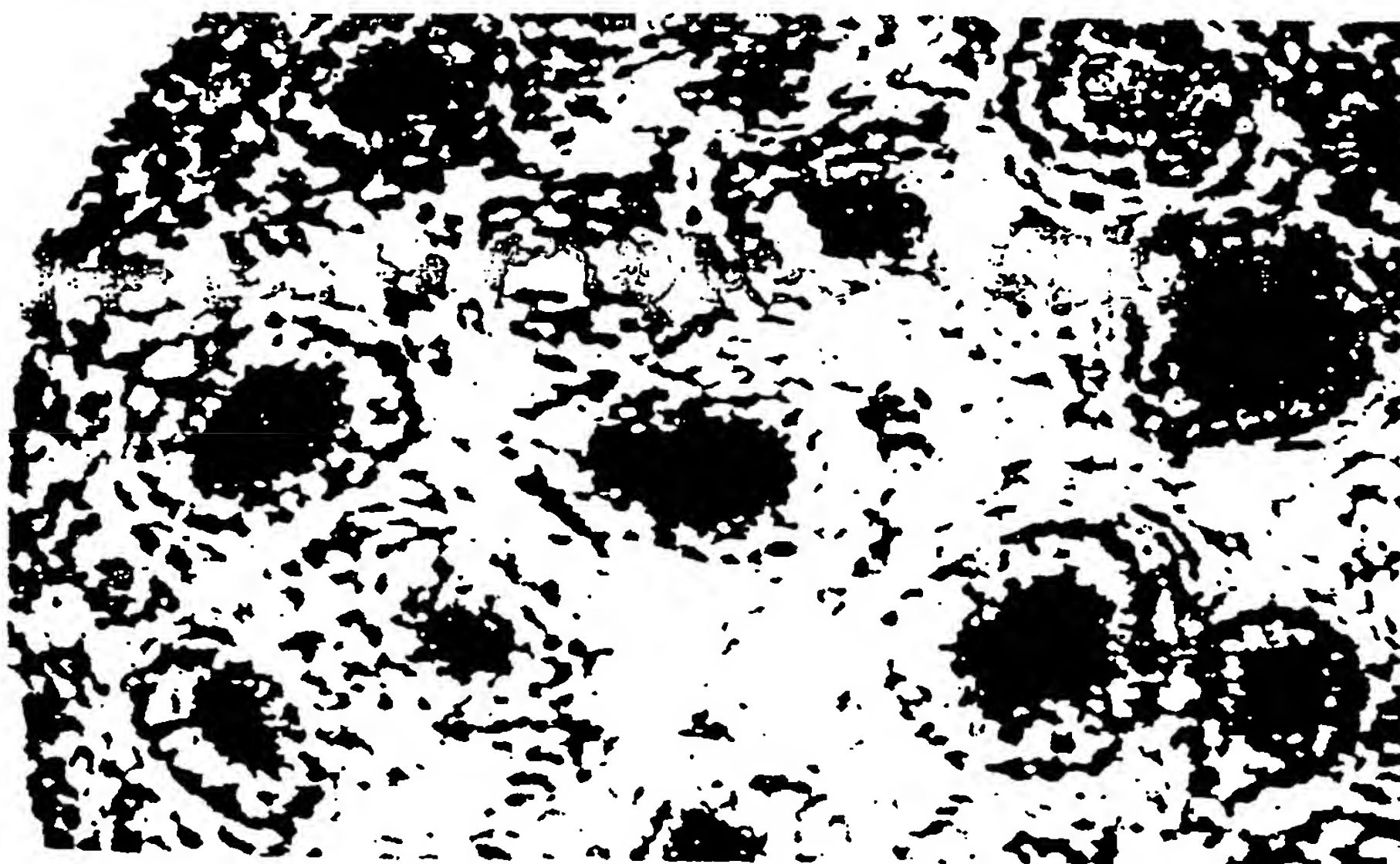


FIG. 9a





FIG. 10a



FIG. 10b



FIG. 11a



FIG. 11b

## --SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (I) APPLICANT: The Johns Hopkins University School of Medicine
- (ii) TITLE OF INVENTION: GROWTH DIFFERENTIATION FACTOR-9
- (iii) NUMBER OF SEQUENCES: 26
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Fish & Richardson
  - (B) STREET: 4225 Executive Square, Suite 1400
  - (C) CITY: San Diego
  - (D) STATE: California
  - (E) COUNTRY: USA
  - (F) ZIP: 92037
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/946,092
  - (B) FILING DATE: 06-OCT-1997
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/491,835
  - (B) FILING DATE: 23-OCT-1995
  - (C) CLASSIFICATION:
- (vi) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: PCT/US94/00685
  - (B) FILING DATE: 12-JAN-1994
  - (C) CLASSIFICATION:
- (vi) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/003,303
  - (B) FILING DATE: 12-JAN-1993
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Lisa A. Haile Ph.D., J.D.
  - (B) REGISTRATION NUMBER: 38,347
  - (C) REFERENCE/DOCKET NUMBER: 07265/138WO1
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (619) 678-5070
  - (B) TELEFAX: (619) 678-5099

## (2) INFORMATION FOR SEQ ID NO:1:

- (I) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:  
(B) CLONE: SJL160

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1..35  
(D) OTHER INFORMATION: /note= "Where "B" occurs, B =  
inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGGAATTCG GBTGGVANVA NTGGRTBRTB KCBCC

35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:  
(B) CLONE: SJL153

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGGAATTCT CADSCRCADC YNBTDGYDRY CAT

33

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1712 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:  
(B) CLONE: GDF-9

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 29..1351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGCGTTCCT TCTTAGTTCT TCCAAGTC ATG GCA CTT CCC AGC AAC TTC CTG  
Met Ala Leu Pro Ser Asn Phe Leu  
1 5

52

TTG Leu	GGG Gly	GTT Val	TGC Cys	TGC Cys	TTT Phe	GCC Ala	TGG Trp	CTG Leu	TGT Cys	TTT Phe	CTT Leu	AGT Ser	AGC Ser	CTT Leu	AGC Ser	100
10	15	20														
TCT Ser	CAG Gln	GCT Ala	TCT Ser	ACT Thr	GAA Glu	GAA Glu	TCC Ser	CAG Gln	AGT Ser	GGA Gly	GCC Ala	AGT Ser	GAA Glu	AAT Asn	GTG Val	148
25	30	35													40	
GAG Glu	TCT Ser	GAG Glu	GCA Ala	GAC Asp	CCC Pro	TGG Trp	TCC Ser	TTG Leu	CTG Leu	CTG Leu	CCT Pro	GTA Val	GAT Asp	GGG Gly	ACT Thr	196
				45				50						55		
GAC Asp	AGG Arg	TCT Ser	GGC Gly	CTC Leu	TTG Leu	CCC Pro	CCC Pro	CTC Leu	TTT Phe	AAG Lys	GTT Val	CTA Leu	TCT Ser	GAT Asp	AGG Arg	244
			60					65					70			
CGA Arg	GGT Gly	GAG Glu	ACC Thr	CCT Pro	AAG Lys	CTG Leu	CAG Gln	CCT Pro	GAC Asp	TCC Ser	AGA Arg	GCA Ala	CTC Leu	TAC Tyr	TAC Tyr	292
	75						80					85				
ATG Met	AAA Lys	AAG Lys	CTC Leu	TAT Tyr	AAG Lys	ACG Thr	TAT Tyr	GCT Ala	ACC Thr	AAA Lys	GAG Glu	GGG Gly	GTT Val	CCC Pro	AAA Lys	340
90	95									100						
CCC Pro	AGC Ser	AGA Arg	AGT Ser	CAC His	CTC Leu	TAC Tyr	AAT Asn	ACC Thr	GTC Val	CGG Arg	CTC Leu	TTC Phe	AGT Ser	CCC Pro	TGT Cys	388
105					110					115					120	
GCC Ala	CAG Gln	CAA Gln	GAG Glu	CAG Gln	GCA Ala	CCC Pro	AGC Ser	AAC Asn	CAG Gln	GTG Val	ACA Thr	GGA Gly	CCG Pro	CTG Leu	CCG Pro	436
				125					130					135		
ATG Met	GTG Val	GAC Asp	CTG Leu	CTG Leu	TTT Phe	AAC Asn	CTG Leu	GAC Asp	CGG Arg	GTG Val	ACT Thr	GCC Ala	ATG Met	GAA Glu	CAC His	484
			140					145					150			
TTG Leu	CTC Leu	AAA Lys	TCG Ser	GTC Val	TTG Leu	CTA Leu	TAC Tyr	ACT Thr	CTG Leu	AAC Asn	AAC Asn	TCT Ser	GCC Ala	TCT Ser	TCC Ser	532
		155					160					165				
TCC Ser	TCC Ser	ACT Thr	GTG Val	ACC Thr	TGT Cys	ATG Met	TGT Cys	GAC Asp	CTT Leu	GTG Val	GTA Val	AAG Lys	GAG Glu	GCC Ala	ATG Met	580
	170					175					180					
TCT Ser	TCT Ser	GGC Gly	AGG Arg	GCA Ala	CCC Pro	CCA Pro	AGA Arg	GCA Ala	CCG Pro	TAC Tyr	TCA Ser	TTC Phe	ACC Thr	CTG Leu	AAG Lys	628
185					190					195					200	
AAA Lys	CAC His	AGA Arg	TGG Trp	ATT Ile	GAG Glu	ATT Ile	GAT Asp	GTG Val	ACC Thr	TCC Ser	CTC Leu	CTT Leu	CAG Gln	CCC Pro	CTA Leu	676
				205				210						215		
GTG Val	ACC Thr	TCC Ser	AGC Ser	GAG Glu	AGG Arg	AGC Ser	ATT Ile	CAC His	CTG Leu	TCT Ser	GTC Val	AAT Asn	TTT Phe	ACA Thr	TGC Cys	724
			220					225					230			
ACA Thr	AAA Lys	GAC Asp	CAG Gln	GTG Val	CCA Pro	GAG Glu	GAC Asp	GGA Gly	GTG Val	TTT Phe	AGC Ser	ATG Met	CCT Pro	CTC Leu	TCA Ser	772
		235					240					245				

GTG	CCT	CCT	TCC	CTC	ATC	TTG	TAT	CTC	AAC	GAC	ACA	AGC	ACC	CAG	GCC		820
Val	Pro	Pro	Ser	Leu	Ile	Leu	Tyr	Leu	Asn	Asp	Thr	Ser	Thr	Gln	Ala		
	250					255					260						
TAC	CAC	TCT	TGG	CAG	TCT	CTT	CAG	TCC	ACC	TGG	AGG	CCT	TTA	CAG	CAT		868
Tyr	His	Ser	Trp	Gln	Ser	Leu	Gln	Ser	Thr	Trp	Arg	Pro	Leu	Gln	His		
	265				270					275					280		
CCC	GGC	CAG	GCC	GGT	GTG	GCT	GCC	CGT	CCC	GTG	AAA	GAG	GAA	GCT	ACT		916
Pro	Gly	Gln	Ala	Gly	Val	Ala	Ala	Arg	Pro	Val	Lys	Glu	Glu	Ala	Thr		
				285					290					295			
GAG	GTG	GAA	AGA	TCT	CCC	CGG	CGC	CGT	CGA	GGG	CAG	AAA	GCC	ATC	CGC		964
Glu	Val	Glu	Arg	Ser	Pro	Arg	Arg	Arg	Arg	Gly	Gln	Lys	Ala	Ile	Arg		
			300					305					310				
TCC	GAA	GCG	AAG	GGG	CCA	CTT	CTT	ACA	GCA	TCC	TTC	AAC	CTC	AGC	GAA		1012
Ser	Glu	Ala	Lys	Gly	Pro	Leu	Leu	Thr	Ala	Ser	Phe	Asn	Leu	Ser	Glu		
		315					320					325					
TAC	TTC	AAA	CAG	TTT	CTT	TTC	CCC	CAA	AAC	GAG	TGT	GAA	CTC	CAT	GAC		1060
Tyr	Phe	Lys	Gln	Phe	Leu	Phe	Pro	Gln	Asn	Glu	Cys	Glu	Leu	His	Asp		
	330					335					340						
TTC	AGA	CTG	AGT	TTT	AGT	CAG	CTC	AAA	TGG	GAC	AAC	TGG	ATC	GTG	GCC		1108
Phe	Arg	Leu	Ser	Phe	Ser	Gln	Leu	Lys	Trp	Asp	Asn	Trp	Ile	Val	Ala		
					350					355					360		
CCG	CAC	AGG	TAC	AAC	CCT	AGG	TAC	TGT	AAA	GGG	GAC	TGT	CCT	AGG	GCG		1156
Pro	His	Arg	Tyr	Asn	Pro	Arg	Tyr	Cys	Lys	Gly	Asp	Cys	Pro	Arg	Ala		
				365					370					375			
GTC	AGG	CAT	CGG	TAT	GGC	TCT	CCT	GTG	CAC	ACC	ATG	GTC	CAG	AAT	ATA		1204
Val	Arg	His	Arg	Tyr	Gly	Ser	Pro	Val	His	Thr	Met	Val	Gln	Asn	Ile		
			380					385					390				
ATC	TAT	GAG	AAG	CTG	GAC	CCT	TCA	GTG	CCA	AGG	CCT	TCG	TGT	GTG	CCG		1252
Ile	Tyr	Glu	Lys	Leu	Asp	Pro	Ser	Val	Pro	Arg	Pro	Ser	Cys	Val	Pro		
		395					400					405					
GGC	AAG	TAC	AGC	CCC	CTG	AGT	GTG	TTG	ACC	ATT	GAA	CCC	GAC	GGC	TCC		1300
Gly	Lys	Tyr	Ser	Pro	Leu	Ser	Val	Leu	Thr	Ile	Glu	Pro	Asp	Gly	Ser		
	410					415					420						
ATC	GCT	TAC	AAA	GAG	TAC	GAA	GAC	ATG	ATA	GCT	ACG	AGG	TGC	ACC	TGT		1348
Ile	Ala	Tyr	Lys	Glu	Tyr	Glu	Asp	Met	Ile	Ala	Thr	Arg	Cys	Thr	Cys		
	425				430					435					440		
CGT	TAGCATGGGG GCCACTTCAA CAAGCCTGCC TGGCAGAGCA ATGCTGTGGG																1401
Arg																	

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 441 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Leu	Pro	Ser	Asn	Phe	Leu	Leu	Gly	Val	Cys	Cys	Phe	Ala	Trp	1	5	10	15
Leu	Cys	Phe	Leu	Ser	Ser	Leu	Ser	Ser	Gln	Ala	Ser	Thr	Glu	Glu	Ser	20	25	30	
Gln	Ser	Gly	Ala	Ser	Glu	Asn	Val	Glu	Ser	Glu	Ala	Asp	Pro	Trp	Ser	35	40	45	
Leu	Leu	Leu	Pro	Val	Asp	Gly	Thr	Asp	Arg	Ser	Gly	Leu	Leu	Pro	Pro	50	55	60	
Leu	Phe	Lys	Val	Leu	Ser	Asp	Arg	Arg	Gly	Glu	Thr	Pro	Lys	Leu	Gln	65	70	75	80
Pro	Asp	Ser	Arg	Ala	Leu	Tyr	Tyr	Met	Lys	Lys	Leu	Tyr	Lys	Thr	Tyr	85	90	95	
Ala	Thr	Lys	Glu	Gly	Val	Pro	Lys	Pro	Ser	Arg	Ser	His	Leu	Tyr	Asn	100	105	110	
Thr	Val	Arg	Leu	Phe	Ser	Pro	Cys	Ala	Gln	Gln	Glu	Gln	Ala	Pro	Ser	115	120	125	
Asn	Gln	Val	Thr	Gly	Pro	Leu	Pro	Met	Val	Asp	Leu	Leu	Phe	Asn	Leu	130	135	140	
Asp	Arg	Val	Thr	Ala	Met	Glu	His	Leu	Leu	Lys	Ser	Val	Leu	Leu	Tyr	145	150	155	160
Thr	Leu	Asn	Asn	Ser	Ala	Ser	Ser	Ser	Ser	Thr	Val	Thr	Cys	Met	Cys	165	170	175	
Asp	Leu	Val	Val	Lys	Glu	Ala	Met	Ser	Ser	Gly	Arg	Ala	Pro	Pro	Arg	180	185	190	
Ala	Pro	Tyr	Ser	Phe	Thr	Leu	Lys	Lys	His	Arg	Trp	Ile	Glu	Ile	Asp	195	200	205	
Val	Thr	Ser	Leu	Leu	Gln	Pro	Leu	Val	Thr	Ser	Ser	Glu	Arg	Ser	Ile	210	215	220	
His	Leu	Ser	Val	Asn	Phe	Thr	Cys	Thr	Lys	Asp	Gln	Val	Pro	Glu	Asp	225	230	235	240
Gly	Val	Phe	Ser	Met	Pro	Leu	Ser	Val	Pro	Pro	Ser	Leu	Ile	Leu	Tyr	245	250	255	
Leu	Asn	Asp	Thr	Ser	Thr	Gln	Ala	Tyr	His	Ser	Trp	Gln	Ser	Leu	Gln	260	265	270	



Ser Thr Trp Arg Pro Leu Gln His Pro Gly Gln Ala Gly Val Ala Ala  
 275 280 285  
 Arg Pro Val Lys Glu Glu Ala Thr Glu Val Glu Arg Ser Pro Arg Arg  
 290 295 300  
 Arg Arg Gly Gln Lys Ala Ile Arg Ser Glu Ala Lys Gly Pro Leu Leu  
 305 310 315 320  
 Thr Ala Ser Phe Asn Leu Ser Glu Tyr Phe Lys Gln Phe Leu Phe Pro  
 325 330 335  
 Gln Asn Glu Cys Glu Leu His Asp Phe Arg Leu Ser Phe Ser Gln Leu  
 340 345 350  
 Lys Trp Asp Asn Trp Ile Val Ala Pro His Arg Tyr Asn Pro Arg Tyr  
 355 360 365  
 Cys Lys Gly Asp Cys Pro Arg Ala Val Arg His Arg Tyr Gly Ser Pro  
 370 375 380  
 Val His Thr Met Val Gln Asn Ile Ile Tyr Glu Lys Leu Asp Pro Ser  
 385 390 395 400  
 Val Pro Arg Pro Ser Cys Val Pro Gly Lys Tyr Ser Pro Leu Ser Val  
 405 410 415  
 Leu Thr Ile Glu Pro Asp Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp  
 420 425 430  
 Met Ile Ala Thr Arg Cys Thr Cys Arg  
 435 440

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 117 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: GDF-3

## (ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..117

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Lys Arg Arg Ala Ala Ile Ser Val Pro Lys Gly Phe Cys Arg Asn Phe  
 1 5 10 15  
 Cys His Arg His Gln Leu Phe Ile Asn Phe Gln Asp Leu Gly Trp His  
 20 25 30  
 Lys Trp Val Ile Ala Pro Lys Gly Phe Met Ala Asn Tyr Cys His Gly  
 35 40 45

Glu Cys Pro Phe Ser Met Thr Thr Tyr Leu Asn Ser Ser Asn Tyr Ala  
 50 55 60  
 Phe Met Gln Ala Leu Met His Met Ala Asp Pro Lys Val Pro Lys Ala  
 65 70 75 80  
 Val Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Met Leu Tyr Gln Asp  
 85 90 95  
 Ser Asp Lys Asn Val Ile Leu Arg His Tyr Glu Asp Met Val Val Asp  
 100 105 110  
 Glu Cys Gly Cys Gly  
 115

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 118 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

- (B) CLONE: GDF-9

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..118

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Phe Asn Leu Ser Glu Tyr Phe Lys Gln Phe Leu Phe Pro Gln Asn Glu  
 1 5 10 15  
 Cys Glu Leu His Asp Phe Arg Leu Ser Phe Ser Gln Leu Lys Trp Asp  
 20 25 30  
 Asn Trp Ile Val Ala Pro His Arg Tyr Asn Pro Arg Tyr Cys Lys Gly  
 35 40 45  
 Asp Cys Pro Arg Ala Val Arg His Arg Tyr Gly Ser Pro Val His Thr  
 50 55 60  
 Met Val Gln Asn Ile Ile Tyr Glu Lys Leu Asp Pro Ser Val Pro Arg  
 65 70 75 80  
 Pro Ser Cys Val Pro Gly Lys Tyr Ser Pro Leu Ser Val Leu Thr Ile  
 85 90 95  
 Glu Pro Asp Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp Met Ile Ala  
 100 105 110  
 Thr Arg Cys Thr Cys Arg  
 115

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 122 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- (vii) IMMEDIATE SOURCE:  
    (B) CLONE: GDF-1

- (ix) FEATURE:  
    (A) NAME/KEY: Protein  
    (B) LOCATION: 1..122

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly Ala  
1                    5                    10                    15  
Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp His  
                    20                    25                    30  
Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly  
                    35                    40                    45  
Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala  
                    50                    55                    60  
Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro Gly  
65                    70                    75                    80  
Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile Ser  
                    85                    90                    95  
Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr Glu  
                    100                    105                    110  
Asp Met Val Val Asp Glu Cys Gly Cys Arg  
                    115                    120

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 118 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- (vii) IMMEDIATE SOURCE:  
    (B) CLONE: Vg-1

- (ix) FEATURE:  
    (A) NAME/KEY: Protein  
    (B) LOCATION: 1..118

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Arg Arg Lys Arg Ser Tyr Ser Lys Leu Pro Phe Thr Ala Ser Asn Ile
1      5      10      15
Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly Trp Gln
20      25      30
Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly
35      40      45
Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn His Ala
50      55      60
Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile Pro Leu
65      70      75      80
Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu Phe Tyr
85      90      95
Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met Ala Val
100      105      110
Asp Glu Cys Gly Cys Arg
115

```

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (vii) IMMEDIATE SOURCE:

(B) CLONE: Vgr-1

## (ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..118

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

Arg Val Ser Ser Ala Ser Asp Tyr Asn Ser Ser Glu Leu Lys Thr Ala
1      5      10      15
Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
20      25      30
Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
35      40      45
Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
50      55      60
Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
65      70      75      80

```

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe  
                             85                            90                            95

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
                             100                            105                            110

Arg Ala Cys Gly Cys His  
                             115

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vii) IMMEDIATE SOURCE:

(B) CLONE: OP-1

## (ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..118

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser Asp Gln Arg Gln Ala  
 1                            5                            10                            15

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln  
                             20                            25                            30

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly  
                             35                            40                            45

Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala Thr Asn His Ala  
                             50                            55                            60

Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Glu Thr Val Pro Lys  
                             65                            70                            75                            80

Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val Leu Tyr Phe  
                             85                            90                            95

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
                             100                            105                            110

Arg Ala Cys Gly Cys His  
                             115

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
(B) CLONE: BMP-5

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Arg	Met	Ser	Ser	Val	Gly	Asp	Tyr	Asn	Thr	Ser	Glu	Gln	Lys	Gln	Ala	1	5	10	15
Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	20	25	30	
Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala	Ala	Phe	Tyr	Cys	Asp	Gly	35	40	45	
Glu	Cys	Ser	Phe	Pro	Leu	Asn	Ala	His	Met	Asn	Ala	Thr	Asn	His	Ala	50	55	60	
Ile	Val	Gln	Thr	Leu	Val	His	Leu	Met	Phe	Pro	Asp	His	Val	Pro	Lys	65	70	75	80
Pro	Cys	Cys	Ala	Pro	Thr	Lys	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe	85	90	95	
Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys	Lys	Tyr	Arg	Asn	Met	Val	Val	100	105	110	
Arg	Ser	Cys	Gly	Cys	His											115			

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 118 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 60A

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser	Pro	Asn	Asn	Val	Pro	Leu	Leu	Glu	Pro	Met	Glu	Ser	Thr	Arg	Ser	1	5	10	15
Cys	Gln	Met	Gln	Thr	Leu	Tyr	Ile	Asp	Phe	Lys	Asp	Leu	Gly	Trp	His	20	25	30	

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser Gly  
           35                          40                          45  
 Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
           50                          55                          60  
 Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro Lys  
   65                          70                          75                          80  
 Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr His  
                           85                          90                          95  
 Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile Val  
                   100                          105                          110  
 Lys Ser Cys Gly Cys His  
                   115

## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 117 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

- (B) CLONE: BMP-2

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..117

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Glu Lys Arg Gln Ala Lys His Lys Gln Arg Lys Arg Leu Lys Ser Ser  
   1                          5                          10                          15  
 Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn  
                   20                          25                          30  
 Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly  
           35                          40                          45  
 Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala  
           50                          55                          60  
 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala  
   65                          70                          75                          80  
 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp  
                           85                          90                          95  
 Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu  
                   100                          105                          110  
 Gly Cys Gly Cys Arg  
                   115



## (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 117 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: BMP-4

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..117

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Arg	Ser	Pro	Lys	His	His	Ser	Gln	Arg	Ala	Arg	Lys	Lys	Asn	Lys	Asn	1	5	10	15
Cys	Arg	Arg	His	Ser	Leu	Tyr	Val	Asp	Phe	Ser	Asp	Val	Gly	Trp	Asn	20	25	30	
Asp	Trp	Ile	Val	Ala	Pro	Pro	Gly	Tyr	Gln	Ala	Phe	Tyr	Cys	His	Gly	35	40	45	
Asp	Cys	Pro	Phe	Pro	Leu	Ala	Asp	His	Leu	Asn	Ser	Thr	Asn	His	Ala	50	55	60	
Ile	Val	Gln	Thr	Leu	Val	Asn	Ser	Val	Asn	Ser	Ser	Ile	Pro	Lys	Ala	65	70	75	80
Cys	Cys	Val	Pro	Thr	Glu	Leu	Ser	Ala	Ile	Ser	Met	Leu	Tyr	Leu	Asp	85	90	95	
Glu	Tyr	Asp	Lys	Val	Val	Leu	Lys	Asn	Tyr	Gln	Glu	Met	Val	Val	Glu	100	105	110	
Gly	Cys	Gly	Cys	Arg												115			

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 118 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: DPP

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..118

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```

Lys Arg His Ala Arg Arg Pro Thr Arg Arg Lys Asn His Asp Asp Thr
1          5          10          15
Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp
          20          25          30
Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly
          35          40          45
Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala
          50          55          60
Val Val Gln Thr Leu Val Asn Asn Met Asn Pro Gly Lys Val Pro Lys
65          70          75          80
Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu
          85          90          95
Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val
          100          105          110
Val Gly Cys Gly Cys Arg
          115

```

## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 119 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: BMP-3

## (ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..119

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```

Gln Thr Leu Lys Lys Ala Arg Arg Lys Gln Trp Ile Glu Pro Arg Asn
1          5          10          15
Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
          20          25          30
Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly
          35          40          45
Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
          50          55          60
Thr Ile Gln Ser Ile Val Arg Ala Val Gly Val Val Pro Gly Ile Pro
65          70          75          80

```

Glu	Pro	Cys	Cys	Val 85	Pro	Glu	Lys	Met	Ser	Ser	Leu	Ser	Ile	Leu 95	Phe
Phe	Asp	Glu	Asn 100	Lys	Asn	Val	Val	Leu 105	Lys	Val	Tyr	Pro	Asn 110	Met	Thr
Val	Glu	Ser 115	Cys	Ala	Cys	Arg									

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 115 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

- (B) CLONE: MIS

(ix) FEATURE:

- ```
(A) NAME/KEY: Protein
(B) LOCATION: 1..115
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

[illegible]

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 121 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
(B) CLONE: Inhibin alpha

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..121

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```

Leu Arg Leu Leu Gln Arg Pro Pro Glu Glu Pro Ala Ala His Ala Asn
1          5          10          15
Cys His Arg Val Ala Leu Asn Ile Ser Phe Gln Glu Leu Gly Trp Glu
          20          25          30
Arg Trp Ile Val Tyr Pro Pro Ser Phe Ile Phe His Tyr Cys His Gly
          35          40          45
Gly Cys Gly Leu His Ile Pro Pro Asn Leu Ser Leu Pro Val Pro Gly
          50          55          60
Ala Pro Pro Thr Pro Ala Gln Pro Tyr Ser Leu Leu Pro Gly Ala Gln
65          70          75          80
Pro Cys Cys Ala Ala Leu Pro Gly Thr Met Arg Pro Leu His Val Arg
          85          90          95
Thr Thr Ser Asp Gly Gly Tyr Ser Phe Lys Tyr Glu Thr Val Pro Asn
          100          105          110
Leu Leu Thr Gln His Cys Ala Cys Ile
          115          120

```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 121 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
(B) CLONE: Inhibin betaA

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..121

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

Arg Arg Arg Arg Arg Gly Leu Glu Cys Asp Gly Lys Val Asn Ile Cys
1          5          10          15
Cys Lys Lys Gln Phe Phe Val Ser Phe Lys Asp Ile Gly Trp Asn Asp
          20          25          30

```

|           |            |     |            |           |     |           |            |     |           |           |     |           |            |           |           |
|-----------|------------|-----|------------|-----------|-----|-----------|------------|-----|-----------|-----------|-----|-----------|------------|-----------|-----------|
| Trp       | Ile<br>35  | Ile | Ala        | Pro       | Ser | Gly       | Tyr<br>40  | His | Ala       | Asn       | Tyr | Cys<br>45 | Glu        | Gly       | Glu       |
| Cys       | Pro<br>50  | Ser | His        | Ile       | Ala | Gly<br>55 | Thr        | Ser | Gly       | Ser<br>60 | Ser | Leu       | Ser        | Phe       | His       |
| Ser<br>65 | Thr        | Val | Ile        | Asn<br>70 | His | Tyr       | Arg        | Met | Arg       | Gly<br>75 | His | Ser       | Pro        | Phe       | Ala<br>80 |
| Asn       | Leu        | Lys | Ser        | Cys<br>85 | Cys | Val       | Pro        | Thr | Lys<br>90 | Leu       | Arg | Pro       | Met        | Ser<br>95 | Met       |
| Leu       | Tyr        | Tyr | Asp<br>100 | Asp       | Gly | Gln       | Asn<br>105 | Ile | Ile       | Lys       | Lys | Asp       | Ile<br>110 | Gln       | Asn       |
| Met       | Ile<br>115 | Val | Glu        | Glu       | Cys | Gly       | Cys<br>120 | Ser |           |           |     |           |            |           |           |

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 120 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Inhibin betaB

(ix) **FEATURE:**

- (A) NAME/KEY: Protein  
(B) LOCATION: 1..120

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

|           |           |            |           |           |     |           |           |            |           |           |           |           |            |           |           |
|-----------|-----------|------------|-----------|-----------|-----|-----------|-----------|------------|-----------|-----------|-----------|-----------|------------|-----------|-----------|
| Arg<br>1  | Ile       | Arg        | Lys<br>5  | Arg       | Gly | Leu       | Glu       | Cys<br>10  | Asp       | Gly       | Arg       | Thr       | Asn<br>15  | Leu       | Cys       |
| Cys       | Arg       | Gln<br>20  | Gln       | Phe       | Phe | Ile       | Asp       | Phe<br>25  | Arg       | Leu       | Ile       | Gly       | Trp<br>30  | Asn       | Asp       |
| Trp       | Ile<br>35 | Ile        | Ala       | Pro       | Thr | Gly       | Tyr<br>40 | Tyr        | Gly       | Asn       | Tyr       | Cys<br>45 | Glu        | Gly       | Ser       |
| Cys       | Pro<br>50 | Ala        | Tyr       | Leu       | Ala | Gly<br>55 | Val       | Pro        | Gly       | Ser       | Ala<br>60 | Ser       | Ser        | Phe       | His       |
| Thr<br>65 | Ala       | Val        | Val       | Asn<br>70 | Gln | Tyr       | Arg       | Met        | Arg       | Gly<br>75 | Leu       | Asn       | Pro        | Gly       | Thr<br>80 |
| Val       | Asn       | Ser        | Cys<br>85 | Cys       | Ile | Pro       | Thr       | Lys        | Leu<br>90 | Ser       | Thr       | Met       | Ser        | Met<br>95 | Leu       |
| Tyr       | Phe       | Asp<br>100 | Asp       | Glu       | Tyr | Asn       | Ile       | Val<br>105 | Lys       | Arg       | Asp       | Val       | Pro<br>110 | Asn       | Met       |

Ile Val Glu Glu Cys Gly Cys Ala  
 115 120

## (2) INFORMATION FOR SEQ ID NO:21:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 114 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (vii) IMMEDIATE SOURCE:

(B) CLONE: TGF-beta1

## (ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..114

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Arg | Ala | Leu | Asp | Thr | Asn | Tyr | Cys | Phe | Ser | Ser | Thr | Glu | Lys | Asn |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Cys | Cys | Val | Arg | Gln | Leu | Tyr | Ile | Asp | Phe | Arg | Lys | Asp | Leu | Gly | Trp |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Lys | Trp | Ile | His | Glu | Pro | Lys | Gly | Tyr | His | Ala | Asn | Phe | Cys | Leu | Gly |
|     |     | 35  |     |     |     |     | 40  |     |     |     | 45  |     |     |     |     |
| Pro | Cys | Pro | Tyr | Ile | Trp | Ser | Leu | Asp | Thr | Gln | Tyr | Ser | Lys | Val | Leu |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Ala | Leu | Tyr | Asn | Gln | His | Asn | Pro | Gly | Ala | Ser | Ala | Ala | Pro | Cys | Cys |
| 65  |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     | 80  |     |
| Val | Pro | Gln | Ala | Leu | Glu | Pro | Leu | Pro | Ile | Val | Tyr | Tyr | Val | Gly | Arg |
|     |     |     | 85  |     |     |     |     | 90  |     |     |     |     |     | 95  |     |
| Lys | Pro | Lys | Val | Glu | Gln | Leu | Ser | Asn | Met | Ile | Val | Arg | Ser | Cys | Lys |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Cys | Ser |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

## (2) INFORMATION FOR SEQ ID NO:22:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 114 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (vii) IMMEDIATE SOURCE:

(B) CLONE: TGF-beta2

(ix) FEATURE:

(A) NAME/KEY: Protein  
(B) LOCATION: 1..114

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```

Lys Arg Ala Leu Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp Asn
1           5           10           15
Cys Cys Leu Arg Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu Gly Trp
          20           25           30
Lys Trp Ile His Glu Pro Lys Gly Tyr Asn Ala Asn Phe Cys Ala Gly
          35           40           45
Ala Cys Pro Tyr Leu Trp Ser Ser Asp Thr Gln His Ser Arg Val Leu
          50           55           60
Ser Leu Tyr Asn Thr Ile Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys
65           70           75           80
Val Ser Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Ile Gly Lys
          85           90           95
Thr Pro Lys Ile Glu Gln Leu Ser Asn Met Ile Val Lys Ser Cys Lys
          100          105          110
Cys Ser

```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 114 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: TGF-beta3

(ix) FEATURE:

(A) NAME/KEY: Protein  
(B) LOCATION: 1..114

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```

Lys Arg Ala Leu Asp Thr Asn Tyr Cys Phe Arg Asn Leu Glu Glu Asn
1           5           10           15
Cys Cys Val Arg Pro Leu Tyr Ile Asp Phe Arg Gln Asp Leu Gly Trp
          20           25           30
Lys Trp Val His Glu Pro Lys Gly Tyr Tyr Ala Asn Phe Cys Ser Gly
          35           40           45

```



Pro Cys Pro Tyr Leu Arg Ser Ala Asp Thr Thr His Ser Thr Val Leu  
 50 55 60

Gly Leu Tyr Asn Thr Leu Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys  
 65 70 75 80

Val Pro Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Val Gly Arg  
 85 90 95

Thr Pro Lys Val Glu Gln Leu Ser Asn Met Val Val Lys Ser Cys Lys  
 100 105 110

Cys Ser

## (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 116 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: TGF-beta4

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..116

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Arg Arg Asp Leu Asp Thr Asp Tyr Cys Phe Gly Pro Gly Thr Asp Glu  
 1 5 10 15

Lys Asn Cys Cys Val Arg Pro Leu Tyr Ile Asp Phe Arg Lys Asp Leu  
 20 25 30

Gln Trp Lys Trp Ile His Glu Pro Lys Gly Tyr Met Ala Asn Phe Cys  
 35 40 45

Met Gly Pro Cys Pro Tyr Ile Trp Ser Ala Asp Thr Gln Tyr Thr Lys  
 50 55 60

Val Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro  
 65 70 75 80

Cys Cys Val Pro Gln Thr Leu Asp Pro Leu Pro Ile Ile Tyr Tyr Val  
 85 90 95

Gly Arg Asn Val Arg Val Glu Gln Leu Ser Asn Met Val Val Arg Ala  
 100 105 110

Cys Lys Cys Ser  
 115

## (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 114 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
(B) CLONE: TGF-beta5

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..114

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```

Lys Arg Gly Val Gly Gln Glu Tyr Cys Phe Gly Asn Asn Gly Pro Asn
1          5          10          15
Cys Cys Val Lys Pro Leu Tyr Ile Asn Phe Arg Lys Asp Leu Gly Trp
          20          25          30
Lys Trp Ile His Glu Pro Lys Gly Tyr Glu Ala Asn Tyr Cys Leu Gly
          35          40          45
Asn Cys Pro Tyr Ile Trp Ser Met Asp Thr Gln Tyr Ser Lys Val Leu
          50          55          60
Ser Leu Tyr Asn Gln Asn Asn Pro Gly Ala Ser Ile Ser Pro Cys Cys
          65          70          75          80
Val Pro Asp Val Leu Glu Pro Leu Pro Ile Ile Tyr Tyr Val Gly Arg
          85          90          95
Thr Ala Lys Val Glu Gln Leu Ser Asn Met Val Val Arg Ser Cys Asn
          100          105          110
Cys Ser

```

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 454 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
(B) CLONE: Human GDF-9

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..454

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Arg | Pro | Asn | Lys | Phe | Leu | Leu | Trp | Phe | Cys | Cys | Phe | Ala | Trp | 1   | 5   | 10  | 15  |
| Leu | Cys | Phe | Pro | Ile | Ser | Leu | Gly | Ser | Gln | Ala | Ser | Gly | Gly | Glu | Ala | 20  | 25  | 30  |     |
| Gln | Ile | Ala | Ala | Ser | Ala | Glu | Leu | Glu | Ser | Gly | Ala | Met | Pro | Trp | Ser | 35  | 40  | 45  |     |
| Leu | Leu | Gln | His | Ile | Asp | Glu | Arg | Asp | Arg | Ala | Gly | Leu | Leu | Pro | Ala | 50  | 55  | 60  |     |
| Leu | Phe | Lys | Val | Leu | Ser | Val | Gly | Arg | Gly | Gly | Ser | Pro | Arg | Leu | Gln | 65  | 70  | 75  | 80  |
| Pro | Asp | Ser | Arg | Ala | Leu | His | Tyr | Met | Lys | Lys | Leu | Tyr | Lys | Thr | Tyr | 85  | 90  | 95  |     |
| Ala | Thr | Lys | Glu | Gly | Ile | Pro | Lys | Ser | Asn | Arg | Ser | His | Leu | Tyr | Asn | 100 | 105 | 110 |     |
| Thr | Val | Arg | Leu | Phe | Thr | Pro | Cys | Thr | Arg | His | Lys | Gln | Ala | Pro | Gly | 115 | 120 | 125 |     |
| Asp | Gln | Val | Thr | Gly | Ile | Leu | Pro | Ser | Val | Glu | Leu | Leu | Phe | Asn | Leu | 130 | 135 | 140 |     |
| Asp | Arg | Ile | Thr | Thr | Val | Glu | His | Leu | Leu | Lys | Ser | Val | Leu | Leu | Tyr | 145 | 150 | 155 | 160 |
| Asn | Ile | Asn | Asn | Ser | Val | Ser | Phe | Ser | Ser | Ala | Val | Lys | Cys | Val | Cys | 165 | 170 | 175 |     |
| Asn | Leu | Met | Ile | Lys | Glu | Pro | Lys | Ser | Ser | Ser | Arg | Thr | Leu | Gly | Arg | 180 | 185 | 190 |     |
| Ala | Pro | Tyr | Ser | Phe | Thr | Phe | Asn | Ser | Gln | Phe | Glu | Phe | Gly | Lys | Lys | 195 | 200 | 205 |     |
| His | Lys | Trp | Ile | Gln | Ile | Asp | Val | Thr | Ser | Leu | Leu | Gln | Pro | Leu | Val | 210 | 215 | 220 |     |
| Ala | Ser | Asn | Lys | Arg | Ser | Ile | His | Met | Ser | Ile | Asn | Phe | Thr | Cys | Met | 225 | 230 | 235 | 240 |
| Lys | Asp | Gln | Leu | Glu | His | Pro | Ser | Ala | Gln | Asn | Gly | Leu | Phe | Asn | Met | 245 | 250 | 255 |     |
| Thr | Leu | Val | Ser | Pro | Ser | Leu | Ile | Leu | Tyr | Leu | Asn | Asp | Thr | Ser | Ala | 260 | 265 | 270 |     |
| Gln | Ala | Tyr | His | Ser | Trp | Tyr | Ser | Leu | His | Tyr | Lys | Arg | Arg | Pro | Ser | 275 | 280 | 285 |     |
| Gln | Gly | Pro | Asp | Gln | Glu | Arg | Ser | Leu | Ser | Ala | Tyr | Pro | Val | Gly | Glu | 290 | 295 | 300 |     |
| Glu | Ala | Ala | Glu | Asp | Gly | Arg | Ser | Ser | His | His | Arg | His | Arg | Arg | Gly | 305 | 310 | 315 | 320 |
| Gln | Glu | Thr | Val | Ser | Ser | Glu | Leu | Lys | Lys | Pro | Leu | Gly | Pro | Ala | Ser | 325 | 330 | 335 |     |

Phe Asn Leu Ser Glu Tyr Phe Arg Gln Phe Leu Leu Pro Gln Asn Glu  
340 345 350  
Cys Glu Leu His Asp Phe Arg Leu Ser Phe Ser Gln Leu Lys Trp Asp  
355 360 365  
Asn Trp Ile Val Ala Pro His Arg Tyr Asn Pro Arg Tyr Cys Lys Gly  
370 375 380  
Asp Cys Pro Arg Ala Val Gly His Arg Tyr Gly Ser Pro Val His Thr  
385 390 395 400  
Met Val Gln Asn Ile Ile Tyr Glu Lys Leu Asp Ser Ser Val Pro Arg  
405 410 415  
Pro Ser Cys Val Pro Ala Lys Tyr Ser Pro Leu Ser Val Leu Thr Ile  
420 425 430  
Glu Pro Asp Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp Met Ile Ala  
435 440 445  
Thr Lys Cys Thr Cys Arg--  
450

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/21199

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/18, 31/185

US CL : 424/85.1, 130.1, 139.1, 141.1, 142.1; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.1, 130.1, 139.1, 141.1, 142.1; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, GenEMBL sequence databases

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                 | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A         | DONG, J. et al. Growth differentiation factor-9 is required during early ovarian folliculogenesis. Nature. 10 October 1996, Vol. 383, pages 531-535, see entire document.                                                          | 1-11                  |
| A         | MASSAGUE, J. The TGF- $\beta$ family of growth and differentiation factors. Cell. March 1987, Vol. 49, pages 437-438, see entire document.                                                                                         | 1-11                  |
| A,P       | FITZPATRICK, S. L. et al. Expression of growth differentiation factor-9 messenger ribonucleic acid in ovarian and nonovarian rodent and human tissues. Endocrinology. 1998, Vol. 139, No. 5, pages 2571-2578, see entire document. | 1-11                  |



Further documents are listed in the continuation of Box C.



See patent family annex.

|                                                                                                                                                                          |                                                                                                                                                                                                                                                   |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * Special categories of cited documents:                                                                                                                                 | * T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention                                              |
| * A* document defining the general state of the art which is not considered to be of particular relevance                                                                | * X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone                                                                     |
| * B* earlier document published on or after the international filing date                                                                                                | * Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| * L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | * A* document member of the same patent family                                                                                                                                                                                                    |
| * O* document referring to an oral disclosure, use, exhibition or other means                                                                                            |                                                                                                                                                                                                                                                   |
| * P* document published prior to the international filing date but later than the priority date claimed                                                                  |                                                                                                                                                                                                                                                   |

Date of the actual completion of the international search

31 DECEMBER 1998

Date of mailing of the international search report

26 JAN 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ELIZABETH C. KEMMERER

Telephone No. (703) 308-0196

**INTERNATIONAL SEARCH REPORT**International application No.  
PCT/US98/21199**Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☒

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/21199

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-6, drawn to a method for inhibiting oocyte maturation comprising administering a GDF-9 antibody.  
Group II, claim(s) 1 and 7, drawn to a method for inhibiting oocyte maturation comprising administering GDF-9 antisense molecule.

Group III, claim(s) 8-11, drawn to a method for inducing an immune response in a subject to GDF-9 comprising administering GDF-9 or a fragment thereof.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical feature of Group I is the active method step of administering a GDF-9 antibody. The other Groups do not require administration of an antibody, and thus do not share a special technical feature with Group I. Furthermore, the agents administered in the methods of Groups II and III have completely different physical and chemical properties.



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